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**THE DEVELOPMENT OF A SYSTEM FOR
ENHANCED RADIONUCLIDE DETECTION**

ANNUAL REPORT

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NOVEMBER 1987

Supported by

**U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012**

Contract No. DAMD17-84-C-4222

**The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205**

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90 10 20 30 40

REPORT DOCUMENTATION PAGE			
1a. REPORT SECURITY CLASSIFICATION Unclassified		1b RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION The Johns Hopkins University School of Medicine		6b. OFFICE SYMBOL (If applicable)	
6c. ADDRESS (City, State, and ZIP Code) Baltimore, Maryland 21205		7a. NAME OF MONITORING ORGANIZATION	
7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)	
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-84-C-4222		10. SOURCE OF FUNDING NUMBERS	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012		PROGRAM ELEMENT NO. 63764A	PROJECT NO. 3M4- 63764D995
		TASK NO. AB	WORK UNIT ACCESSION NO. 161
11. TITLE (Include Security Classification) (U) The Development of a System for Enhanced Radionuclide Detection			
12. PERSONAL AUTHOR(S) John K. Frost, Karen E. Warden, John K. Frost, Jr., Isaac N. Bankman, Norman J. Pressman, Prabodh K. Gupta, Darrell L. Cook, G.W. Gill, R.L. Showers, R.K. Traub			
13a. TYPE OF REPORT Annual		13b. TIME COVERED FROM 8/1/85 TO 7/31/86	14. DATE OF REPORT (Year, Month, Day) 1987 November
15. PAGE COUNT			
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Autoradiography; Computer; Detection; Energy Transfer, Fluorochrome; Histology; Image Analysis; Image Intensification; Instrumentation; Photonic, Photo-limited Imaging	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Frequently, toxicologic processes involve concentrations of biologically active substances so low that to be detected, localized, and quantified they require attachment of appropriate radiolabels and subsequent autoradiography exposure periods that may extend for many months. The major objective of this research is to develop and deliver a system to reduce this long data collection period while maintaining the advantage of spatial resolution inherent in autoradiography. After completing concept validation and after careful testing of three low-light-level camera system which were submitted by vendors for evaluation, a photon-limited camera and imaging system by Hamamatsu and a programmable image display unit by Gould were selected and ordered for purchase. Software programs to distinguish and analyze microstructures and their properties were generated.			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21 ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian		22b TELEPHONE (Include Area Code) 301-663-7325	22c. OFFICE SYMBOL SGRD-RMI-S

SUMMARY

Frequently, toxicologic processes involve concentrations of biologically active substances so low that to be detected, localized, and quantified they require attachment of appropriate radiolabels and subsequent autoradiography exposure periods that may extend for many months. The major objective of this research is to develop and deliver a system to reduce this long data collection period while maintaining the advantage of spatial resolution inherent in autoradiography.

After completing concept validation and after careful testing of three low light level camera systems which were submitted by vendors for evaluation, a photon-limited camera and imaging system by Hamamatsu and a programmable image display unit by Gould were selected and ordered for purchase. Software programs to distinguish and analyze microstructures and their properties were generated.



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Availability Codes	
Dist	Avail and/or Special
A-1	

FOREWORD

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I. INTRODUCTION

The U.S. Army Medical Research and Development Command has a need to measure and accurately localize attogram concentrations of chemical compounds in animal and human tissues. Indirect enhancement of the specific energies of the molecules of these compounds is a fundamental approach to this problem.⁵⁹

Enhancement involves the substitution of elements in the compound with their radioactive isotopes during synthesis. Less energetic radioactive isotopes, such as ^{14}C (0.156 MeV) and ^3H (0.01861 MeV), do not present difficult specimen handling problems and have half-lives of 5,570 and 12.3 years, respectively. They permit well-designed biological experiments to be conducted. The observed mode of decay for both ^{14}C and ^3H is β -emission. Depending upon the isotope being used, current methods require contact autoradiographic exposures of as much as 6 months to one year duration to record a usable image.⁹⁰

Scintillation detection and counting by this method will be a more effective alternative, for detecting weak β -emitting radionuclides, than autoradiography. In this method, the energy of the β -particles excites a scintillator, which converts β -particle kinetic energy into potentially detectable photons. In this current effort, these photonic events are temporally and spatially accumulated as a function of position. The problem is being approached by demonstrating the feasibility of rapidly and microscopically imaging low-concentration radiolabeled tissues by developing: (1) methods for converting the energy of emitted β -particles to photons and (2) working instrumentation for recording the resulting scintillations with high spatial and temporal resolution.^{12, 23, 31, 60, 91, 97}

II. MATERIALS AND METHODS

A. Data Acquisition and Processing Systems

There are two major items of equipment whose purchases are essential for this contractual effort:

- 1) The Gould IP8400 Programmable Image Display Unit*, and
- 2) The Hamamatsu C1966-20 Video-Intensified Microscopy System (VIM)**.

While much of the thorough evaluation of each of these items was carried out during the previous year, it was completed in this year with decision to purchase. A request for approval was granted, and the order for this equipment was placed May 23, 1986.

B. Development of Computer Software for Data Analysis

The program Particlet was obtained from our USAMRICD collaborator, R.K. Traub. The primary function of Particlet is to enumerate and to calculate the area of microstructures having intensity values above or below a user-supplied threshold. The results displayed to the user are:

- 1) threshold value,
- 2) particle count, and
- 3) total integrated area of counted particles.

In this context, a "particle" is defined as a contiguous set of pixels whose values all exceed or equal some threshold, and which are surrounded entirely by pixels whose values are all below that threshold.

Two modifications had to be made before Particlet could be incorporated onto our DEC VAX 11/780 system, worked upon to develop the data analysis program, and delivered to the Army in the format required on completion of the contract:

- 1) The first required the alteration of program code, so that image data were read in the form of byte-packed 8-bit data and then converted to the program format of 64-bit data.
- 2) The second was to increase the system working disk space to fifty thousand pages, because of the large virtual memory.

* Gould, Inc., San Jose, CA

** Photonic Microscopy, Inc., Oakbrook, IL

A program called Microstructural Analysis Routine (MSAR) was then developed for this project by our computer scientist. This new program uses Particlet as a basic concept model and is written to analyze data for which Particlet had not been designed. MSAR thus offers a different data analysis and output of the extremely limited photon events (Section III. B.).

III. RESULTS

A. Data Acquisition and Processing Systems

1. Gould IP8400 Programmable Image Display Unit

The Gould IP8400 is a programmable digital image array processing and display unit. It contains look-up tables and special hardware processors to allow user manipulation of stored images and Random Access Memory (RAM) for image storage. In its present configuration it is attached to a VAX-11/780, as its host computer, as a peripheral device on the DEC Unibus.

Image data, acquired from the Hamamatsu C1966 VIM processor by the VAX-11/780, are transferred to the IP8400 RAM at a minimum rate of 1.5 Mbytes/sec. Currently the IP8400 has over 524 Kbytes of RAM in two 512 x 512 x 8-bit memory channels and in look-up tables and registers. Two users can access the IP8400 concurrently, but memory cannot be shared. Each display channel consists of one memory channel interfaced to a Video Output Controller (VOC) capable of driving a monochrome monitor or an RGB color monitor with composite video signals.

The master timing of the IP8400 is selectable and can be changed by software calls to internal registers. Each display channel can be manipulated by an associated joystick and has an alphanumeric overlay generator for producing annotation on the display monitor.

The IP8400 is also equipped with a high-speed pipeline processor, the Digital Video Processor (DVP), which will operate on a combination of 512 x 512 x 8-bit memories. It allows real-time operations such as multiply, add, subtract, compare, and reciprocal divide.

2. Hamamatsu C1966-20 Video-Intensified Microscopy System (VIM)

This is an extremely sensitive system developed for use in ultra low-light-level situations. Its characteristics include a wide dynamic range (to image over different intensity levels), high spatial resolution, a high signal-to-noise ratio, and real time data acquisition capabilities.

After evaluating three low-light-level cameras in the first year (Zeiss Venus, Dage-MTI, and the Hamamatsu C1966-20) and final system evaluations in this second year, the Hamamatsu C1966-20 VIM was chosen for purchase on the basis of its sensitivity and photon-limited characteristics.

The system converts emitted photons into a proportional number of electrons through the use of a two-dimensional photon counting tube that consists of three parts. On one face is a photocathode, where the initial optical image is focused. The photons are converted to electrons here and

subsequently accelerated to the second part of the counting tube, a two-stage microchannel plate (MCP), where they are amplified by electron avalanching. The MCP has two important qualities:

- a) due to its design, the spatial integrity of the image is maintained and
- b) because there is an adjustable gain setting, image intensifier gain (avalanching) can be controlled by the user.

The gain of the system can be operated in an analog mode, if sufficient light is present, or in digital mode for low-light-level imaging. Once the amplified electrons strike the phosphor screen that follows the MCP, a resultant image is presented to an optically coupled low lag vidicon. This produces signal pulses which may then be discriminated, with an acceptance threshold, to eliminate thermal noise that may occur.

The resolution approaches that of a Silicon Intensified Target (SIT) camera (300 to 350 T/ lines as compared with 400 for the SIT). By selecting the type of intensifier and associated photocathode, a range of spectral sensitivity from 140 to 850 nm is possible.

The following is a list of the selected components:

- a) Specially selected ultra-low noise/high resolution VIM photon counting camera head with optional Peltier cooling system. The Peltier cooling system was evaluated to determine its effectiveness in minimization of thermal noise. In addition, vendor specifications indicate that a dark count range of 5 to 10 cps can be achieved by carefully selecting the image intensifier tube.
- b) VIM Controller and control keyboard. To reduce the number of incident light sources and thereby assure a light-tight laboratory environment, the keyboard has been adapted with a disable switch.
- c) DEC DMA (direct memory access) interface and software. This feature directly couples the C1966-20 to the DEC VAX 11/780 computer memory, allowing digitized image data or control/command language to be transferred in either direction between the two devices. It offers the advantage of reducing the amount of user interaction necessary for operation of routine functions.

B. Development of Computer Software for Data Analysis

The Microstructural Analysis Routine (MSAR) was developed to enhance the performance of the USAMRICD Program Particlet (Section II.B.), by providing additional functionality, in order to meet the need to quantitatively assess images derived from radionuclide-labeled sections obtained by the System under development. MSAR (Appendix A and Appendix B) has the ability to perform the following five functions:

1. Read Image Data into VAX 11/780 Memory
2. Define Region of Interest (ROI)
3. Select Threshold
4. Determine Adjacency Criteria
5. Calculate Microstructure Statistics

1. Read Image Data into VAX 11/780 Memory

The user may read a monochromatic, 512 x 512 pixel image from disk into VAX memory. The user is also requested to supply the physical dimension of the pixel in microns, if it is known. This factor is important in calculating the areas and perimeters of the microstructures.

2. Define Region of Interest (ROI)

A region of interest (ROI), which is defined by the user, is a subset of the full field image on which microstructure analysis is performed. If no region of interest is specified, the entire image field is used. The user selects an ROI by entering the X, Y coordinate values for the upper left and lower right corners of the region.

3. Select Threshold

Three types of thresholding specifications are available for user selection:

- a) Minimum Threshold — all pixel values BELOW this value will be ignored during analysis,
- b) Maximum Threshold — all pixel values ABOVE this value will be ignored during analysis,
- c) Threshold Range — a combination of the above specifications such that only pixel values lying BETWEEN the minimum AND maximum, inclusively, will be used in analysis.

These selected thresholds must be values between 0 and 255 to be valid. In addition, the user is given the option of specifying a threshold increment for both the minimum and maximum threshold, either individually or together. This allows analysis to be performed in sequential steps automatically, thereby eliminating user interaction for repetitive operations.

4. Determine Adjacency Criteria

The user is given two choices in determining the criteria for contiguity of adjacent points: 1. Orthogonal; 2. Orthogonal and diagonal. If no adjacency mode is selected, the default is to number two.

5. Calculate Microstructure Statistics

MSAR performs a variety of microstructure calculations. For each specified threshold range of an image, the following are calculated:

- a) Total area of all microstructures (in pixels)
- b) Number of microstructures
- c) Average object area
- d) Average X,Y center of region of interest
- e) Average X,Y weighted center of region of interest
- f) Total number of perimeter points
- g) Average number of perimeter points per object
- h) Total perimeter length
- i) Average perimeter length
- j) Average horizontal and vertical feret diameters
- k) Cumulative and average (per object) integrated optical densities
- l) Average maximum optical density (per object)

The perimeter is calculated in two ways. The first, the total number of perimeter points, is the sum of all of the points on the boundary of the microstructure. The second, the total perimeter length, is the distance travelled, in pixels (or microns if the user supplied a pixel to micron conversion factor), of one complete trace of the perimeter. Once the microstructures have been uniquely labeled, the following statistical calculations are performed for each microstructure within a particular threshold range:

- a) X,Y location of the geometric center
- b) X,Y weighted center
- c) Area
- d) Total number of perimeter points and length
- e) Maximum horizontal and vertical diameters
- f) Integrated optical density (pixel values)
- g) Average integrated optical density (pixel values)
- h) Maximum optical density (pixel values)

Many of the calculation results from the preceding lists are given in units of length. For this reason, the pixel size is requested at the onset of the program. The results obtained will be more meaningful when MSAR is allowed to make calculations using actual dimensions.

The hardcopy results obtained upon the completion of Particlet and MSAR processing of the Experimental Image #ED0030 (Figure 1.) are given in Tables 1 and 2. While each routine offers the number of microstructures and the average area of these within a threshold range (or "slice level"), it is evident from this data that the results from MSAR are more extensive and useful. The information concerning averages is helpful in determining general data trends. For instance, the average weighted X and Y center can direct the user to the region of highest intensity, which corresponds to the region with the highest concentration of radiolabeled substance.

An additional level of detail in MSAR is illustrated in Table 3. Here information about specific microstructures is available for each unique microstructure in the image.

FIGURE 1. Concept Validation Image #ED0030 for Signal Dosimetry Experiment with One-second Summation Time.

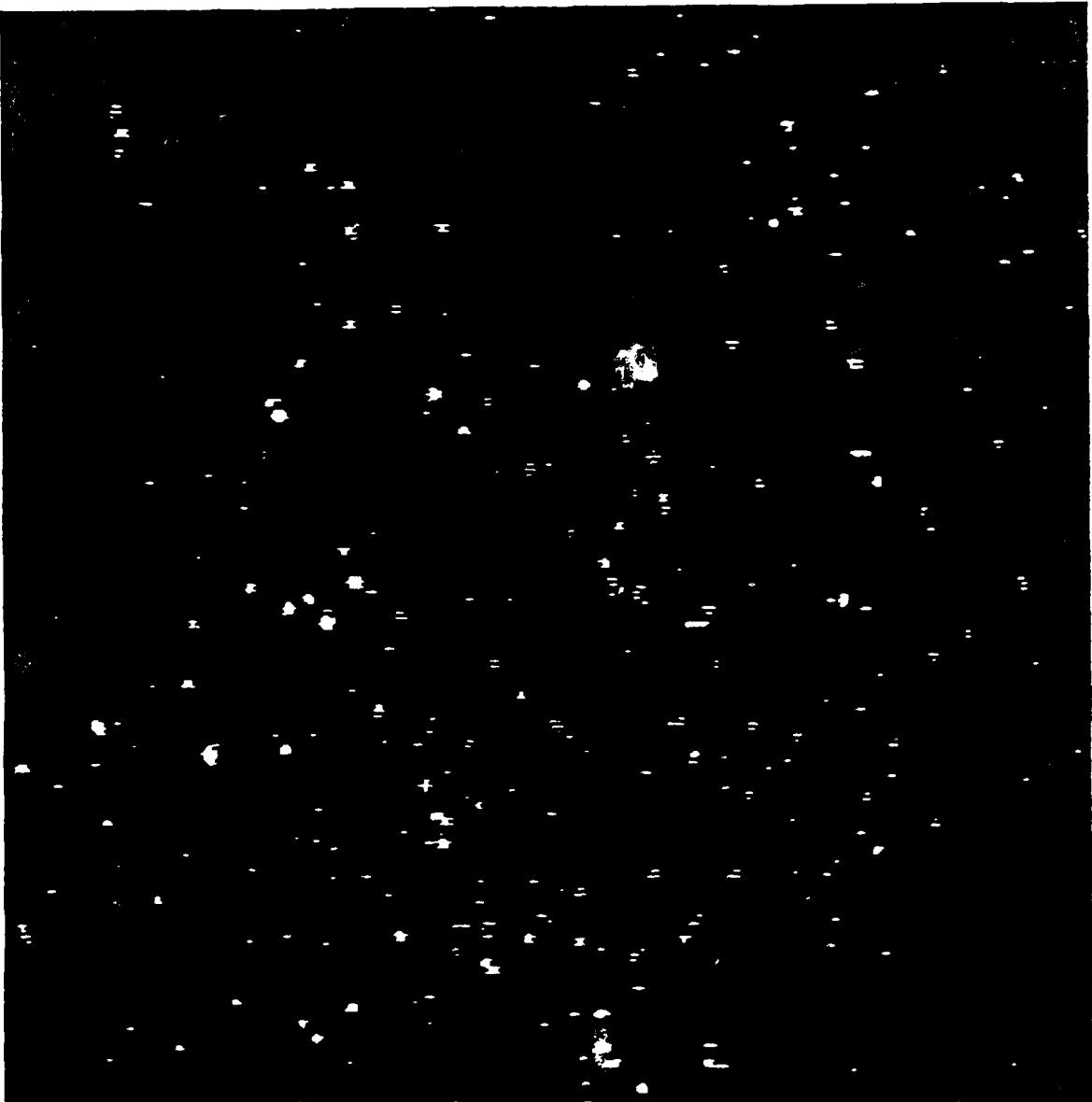


TABLE 1. Tabulated Results from the Particlet Processing of Experimental Image #ED0030

ED0030.DAT

slice level, number, area of particles		
10.00000	1	248303
20.00000	345	1246
30.00000	16	47
40.00000	4	15
50.00000	2	12
60.00000	2	12
70.00000	2	12
80.00000	2	11
90.00000	2	10
100.00000	2	10
110.00000	2	8
120.00000	2	6
130.00000	2	6
140.00000	2	5
150.00000	2	5
160.00000	2	4
170.00000	2	4
180.00000	2	4
190.00000	2	4
200.00000	1	1
210.00000	0	0
220.00000	0	0
230.00000	0	0
240.00000	0	0
250.00000	0	0

TABLE 2. Tabulated Results from the MSAR Processing of Experimental Image #ED0030

FILENAME = ED0030.DAT									
REGION OF INTEREST = TOP LEFT (X= 1, Y= 1), BOTTOM RIGHT (X=512, Y=512)		NUMBER OBJECTS (MICROSTRUCTURES)	AVERAGE OBJECT AREA	AVERAGE X- CENTER	AVERAGE Y- CENTER	AVERAGE WEIGHTED X- CENTER	AVERAGE WEIGHTED Y- CENTER	AVERAGE WEIGHTED Z- CENTER	
THRESHOLD RANGE	TOTAL AREA								
20-255	1246	345	3.61	255.19	303.44	224.48	309.66	309.66	
THRESHOLD RANGE	TOTAL PERIMETER POINTS	AVERAGE PERIMETER POINTS PER OBJECT	TOTAL PERIMETER	AVERAGE PERIMETER PER OBJECT	AVERAGE HORIZONTAL FERET DIAMETER	AVERAGE VERTICAL FERET DIAMETER	CUMULATIVE IOD	AVERAGE IOD PER OBJECT	AVERAGE ODDSET PER OBJECT
20-255	287	0.83	346.00	1.00	2.44	1.49	31036	89.76	29.80

TABLE 3. Tabulated Results for a Particular Threshold Range from
MSAR Processing of Experimental Image #ED0030 (Abridged)

FILENAME = ED0030.CRC_235.MST LOWER THRESHOLD = 20 UPPER THRESHOLD = 235
343 UNIQUE MICROSTRUCTURES FOUND
DEFAULT PIXEL SIZE = 1.000 MICRONS FLAG = 1

UNIQUE MICROSTRUCTURE	WEIGHTED CENTER		WEIGHTED CENTER		AREA COUNT	PERIMETER LENGTH	MAXIMUM HORIZONTAL RUN-LENGTH	MAXIMUM VERTICAL RUN-LENGTH	AVERAGE		
	X CENTER	Y CENTER	X CENTER	Y CENTER					IOC IOC	IOC IOC	IOC IOC
1	223.00	27.43	222.46	27.45	6.00	-1.00	9	2	212	26.56	30
2	223.00	30.00	222.46	30.00	9.00	-1.00	9	1	75	22.00	28
3	54.30	37.00	54.46	37.00	2.00	-1.00	1	1	51	21.50	29
4	54.00	39.00	53.46	39.00	3.00	-1.00	1	1	27	21.66	28
5	106.00	39.00	106.00	39.00	1.00	-1.00	1	1	20	21.33	25
6	220.30	39.00	220.47	39.00	6.00	-1.00	1	1	42	21.33	25
7	313.30	42.00	314.56	42.00	6.00	-1.00	1	1	66	21.00	27
8	108.00	43.00	107.80	43.00	3.00	-1.00	1	1	57	20.66	24
9	243.00	43.00	242.85	43.00	3.00	-1.00	1	1	46	21.33	25
10	176.30	44.00	176.56	44.00	2.00	-1.00	1	1	46	21.00	27
11	106.00	45.00	105.49	45.00	5.00	-1.00	1	1	74	21.33	25
12	238.30	45.00	238.50	45.00	2.00	-1.00	1	1	44	21.00	27
13	138.30	46.00	138.44	46.00	2.00	-1.00	2	1	45	21.33	25
14	206.30	49.00	206.49	49.00	4.00	-1.00	4	1	87	21.25	24
15	248.30	52.00	248.49	52.00	2.00	-1.00	2	1	44	20.66	24
16	249.00	74.00	246.97	74.00	2.00	-1.00	3	1	72	21.00	26
17	424.30	74.00	424.43	74.00	2.00	-1.00	1	1	44	20.00	26
18	169.30	79.00	169.53	79.00	2.00	-1.00	2	1	45	20.00	24
19	290.00	79.00	289.94	79.00	2.00	-1.00	3	1	70	20.33	25
20	237.30	80.00	237.44	80.00	2.00	-1.00	3	1	43	20.33	23
21	290.00	81.00	289.94	81.00	2.00	-1.00	3	1	70	20.33	23
22	346.30	82.00	346.47	82.00	1.00	-1.00	4	1	240	21.00	24
23	213.30	89.00	213.53	89.00	2.00	-1.00	2	1	43	20.33	24
24	229.00	92.00	238.93	92.00	2.00	-1.00	3	1	70	20.33	23
25	162.00	97.00	162.00	97.00	2.00	-1.00	1	1	20	20.00	20
26	344.00	99.00	344.00	99.00	1.00	-1.00	1	1	21	21.00	21
27	336.00	103.00	332.93	103.00	2.00	-1.00	3	1	70	20.33	23
28	306.30	104.00	304.49	104.00	2.00	-1.00	3	1	47	20.33	24
29	343.30	110.00	322.56	110.00	2.00	-1.00	2	1	45	20.33	23
30	417.00	111.00	417.00	111.00	1.00	-1.00	1	1	20	20.00	20
31	243.30	112.00	292.46	112.00	2.00	-1.00	1	1	44	19.00	21
32	417.30	113.00	417.24	113.00	2.00	-1.00	1	1	44	20.00	23
33	266.00	114.00	295.96	114.00	2.00	-1.00	1	1	44	20.33	24
34	390.00	121.00	384.94	121.00	2.00	-1.00	1	1	49	20.00	23
35	280.50	123.00	280.50	123.00	2.00	-1.00	1	1	50	21.00	23
36	29.30	126.00	29.49	126.00	2.00	-1.00	1	1	49	21.00	23
37	89.00	127.00	89.02	127.00	2.00	-1.00	1	1	81	21.00	21
38	28.50	129.00	28.58	129.00	2.00	-1.00	1	1	45	21.00	23
39	88.50	129.00	88.46	129.00	4.00	-1.00	4	1	125	20.75	28
40	356.40	133.10	354.98	133.04	10.00	-1.00	11	4	111	20.00	20
41	41.17	137.08	91.10	137.17	12.00	11	14	1	47	21.00	27
42	358.50	142.00	358.21	142.00	2.00	-1.00	1	1	47	21.00	24
43	390.00	142.00	306.00	142.00	1.00	-1.00	1	1	44	21.00	24
44	90.50	142.00	90.45	142.00	2.00	-1.00	1	1	44	21.00	24
45	89.00	146.00	89.00	146.00	1.00	-1.00	1	1	29	21.00	23

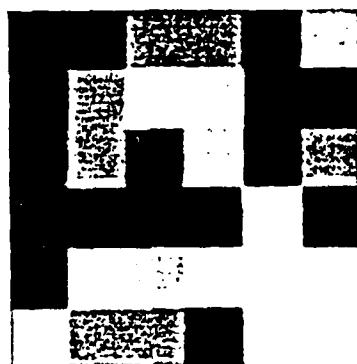
To examine the integrity and accuracy of the Microstructural Analysis Routine, a test image was synthesized (Figure 2).

FIGURE 2. Microstructural Analysis Routine Test Image

Figure 2a. Digital Representation

10	10	20	20	0	30
0	20	50	40	10	0
10	20	10	30	10	20
0	10	10	0	40	0
0	30	30	40	50	30
30	20	20	0	40	40

FIGURE 2b. Analog Representation from Monitor



This test image consists of a six by six matrix containing multiples of six different numerical (pixel) values ranging from zero to fifty in increments of ten. These values were arranged so that, as MSAR processed the image (first by analyzing the 0 to 255 threshold range, then 10 to 255, and so on, until the lower threshold value was equal to fifty), the pixels that fell within the threshold range formed test patterns representing the various conditions of pixel contiguity (Figure 3). Table 4 shows the results of MSAR execution on the test matrix image.

FIGURE 3. Test Patterns of Pixel Contiguity for Different Threshold Ranges of the Microstructure Detection Test Image

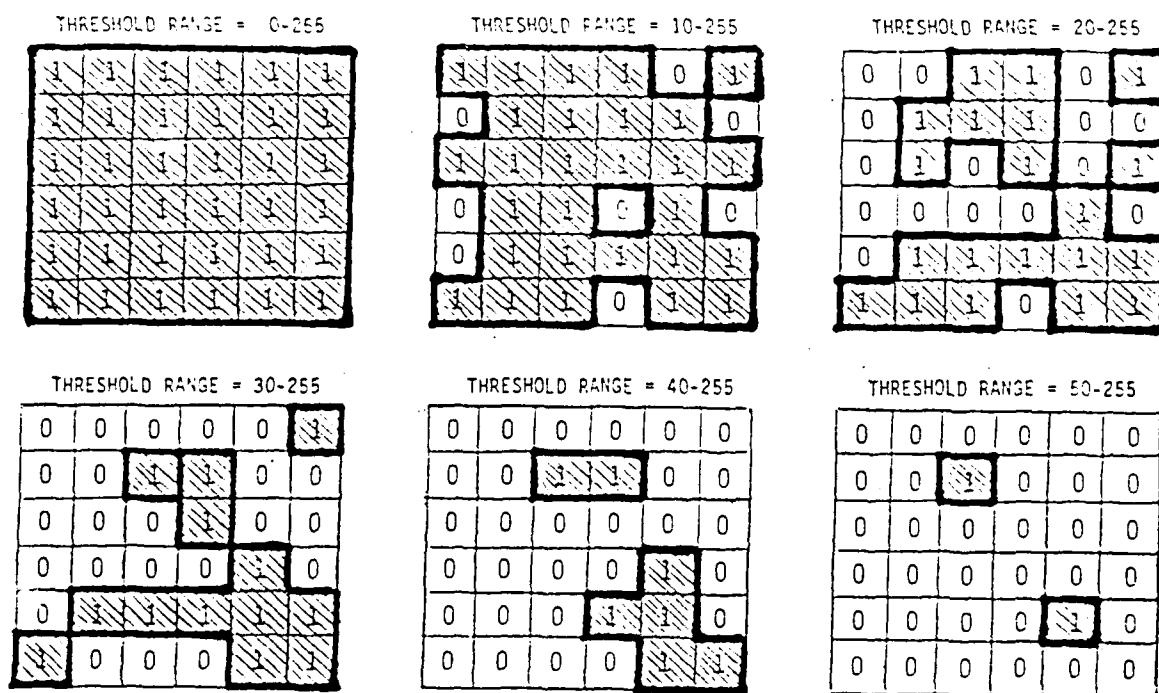


TABLE 4. Tabulated Results from MSAR Processing of Microstructure Detection Test Image

Table 4a

FILENAME = T5T0BJ.DAT
REGION OF INTEREST = TOP LEFT X= 1.Y= 11. BOTTOM RIGHT X= 6.Y= 61

THRESHOLD RANGE	TOTAL AREA	NUMBER OF OBJECTS (MICROSTRUCTURES)	AVERAGE OBJECT AREA	AVERAGE X CENTER	AVERAGE Y CENTER	AVERAGE WEIGHTED X CENTER	AVERAGE WEIGHTED Y CENTER
0-255	36	1	36.00	3.30	3.30	3.30	3.30
10-255	26	2	14.00	4.70	6.30	5.25	5.45
20-255	20	4	5.00	4.74	5.84	5.76	5.99
30-255	12	4	3.25	3.79	5.41	4.17	4.22
40-255	7	2	3.50	4.23	5.86	4.25	4.24
50-255	5	2	1.00	4.00	5.30	4.00	3.95
60-255	0	0	0.00	0.00	0.00	0.00	0.00
70-255	0	0	0.00	0.00	0.00	0.00	0.00
80-255	0	0	0.00	0.00	0.00	0.00	0.00
90-255	0	0	0.00	0.00	0.00	0.00	0.00
100-255	0	0	0.00	0.00	0.00	0.00	0.00
110-255	0	0	0.00	0.00	0.00	0.00	0.00
120-255	0	0	0.00	0.00	0.00	0.00	0.00
130-255	0	0	0.00	0.00	0.00	0.00	0.00
140-255	0	0	0.00	0.00	0.00	0.00	0.00
150-255	0	0	0.00	0.00	0.00	0.00	0.00
160-255	0	0	0.00	0.00	0.00	0.00	0.00
170-255	0	0	0.00	0.00	0.00	0.00	0.00
180-255	0	0	0.00	0.00	0.00	0.00	0.00
190-255	0	0	0.00	0.00	0.00	0.00	0.00
200-255	0	0	0.00	0.00	0.00	0.00	0.00
210-255	0	0	0.00	0.00	0.00	0.00	0.00
220-255	0	0	0.00	0.00	0.00	0.00	0.00
230-255	0	0	0.00	0.00	0.00	0.00	0.00
240-255	0	0	0.00	0.00	0.00	0.00	0.00
250-255	0	0	0.00	0.00	0.00	0.00	0.00

Table 4b
(continuation, to the right, of Table 4a)

THRESHOLD RANGE	TOTAL PERIMETER POINTS	AVERAGE PERIMETER POINTS PER OBJECT	TOTAL PERIMETER	AVERAGE PERIMETER PER OBJECT	AVERAGE HORIZONTAL FERET DIAMETER	AVERAGE VERTICAL FERET DIAMETER	CUMULATIVE IOD	AVERAGE IOD PER OBJECT	AVERAGE OBIET PER OBJECT
0-255	20	20.00	20.00	20.00	4.00	4.00	700	700.00	50.00
10-255	23	11.50	28.00	14.00	3.50	3.30	700	350.00	45.00
20-255	0	0.00	0.00	0.00	2.50	2.00	620	155.00	37.50
30-255	0	0.00	0.00	0.00	2.25	1.75	460	120.00	40.00
40-255	0	0.00	0.00	0.00	2.00	2.00	300	150.00	50.00
50-255	0	0.00	0.00	0.00	1.00	1.00	100	30.00	30.00
60-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
70-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
80-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
90-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
100-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
110-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
120-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
130-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
140-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
150-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
160-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
170-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
180-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
190-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
200-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
210-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
220-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
230-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
240-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
250-255	0	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00

C. Literature Review

Emphasis has been placed on generating and updating a cumulative bibliography pertaining to this project (page 27). For literature to be included in the bibliography, it must first satisfy one or more of the following criteria:

1. Alternative and comparable approaches to autoradiography, such as high-speed autoradiography and scintillation autoradiography.
2. Physical modelling, such as the energy transfer between a radionuclide and scintillant, and single photon detection and capture.
3. Specimen preparation techniques, such as animal tissue and glass-slide preparation, availability and assortment of radionuclides and scintillants, and techniques of scintillation counting.
4. Video intensified microscopy, such as photodetectors and their available technology (photomultiplier tubes, charge-coupled devices, microchannel plates), signal processing (noise analysis and filtering), image processing and enhancement, and the analysis, display and communications of data.

IV. DISCUSSION

The Microstructural Analysis Routine (MSAR) was designed and implemented to address a set of experimental needs for this work. Specifications required that program execution time be minimized without additional computing hardware, and that two types of output files be created for each specified threshold range.

Upon program execution, one file contains an array of the uniquely labeled microstructures and, in addition, any information concerning the creation of the file or necessary for image display. Another file contains the calculated statistics for each unique microstructure. A third file is generated which gives the total and average microstructure statistics for the threshold ranges of a particular image. Additional specifications pertaining to the analyses performed by MSAR were also stipulated:

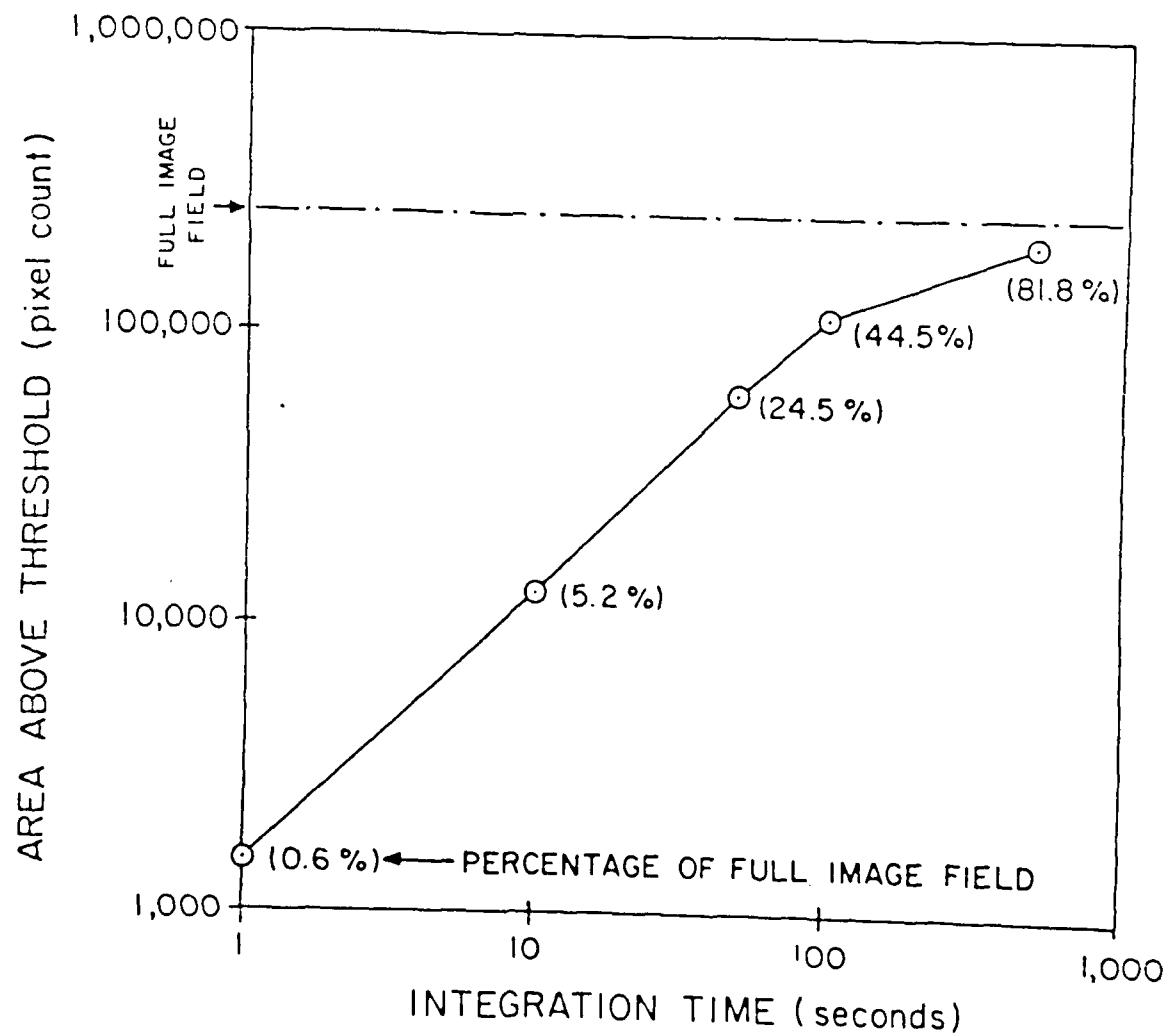
1. Read Image Data into VAX 11/780 Memory
2. Define Region of Interest (ROI)
3. Select Threshold
4. Determine Adjacency Criteria
5. Calculate Microstructure Statistics

These are described in detail in Section III. B.

A test image containing various patterns, created by varying pixel intensity levels within a matrix, was produced to examine the accuracy of the Microstructural Analysis Routine. These patterns simulated microstructures with edge points on the matrix boundary, with diagonal connections, and with holes. Results indicate that microstructures are being detected and analyzed as designed.

Using MSAR, the linearity of the system response as a function of integration time was assessed (Figure 4). In this plot the pixel count above threshold is shown as a function of the integration time. The ratio of photoevent area to full image field is given as a percentage and is printed adjacent to each data point. There is a linearity of the relationship for unsaturated images (i.e., when less than 44.5% of the image field is affected) indicating that, for a fixed source, the integration time is proportional to the cumulative area of the detected photoevents, until saturation effects alter this correlation due to overlapping events. Saturation begins to affect this linear relationship once the photoevent density reaches a concentration greater than 44.5% of the image field (Figure 4). At this value, continuing accumulation results in a progressively higher rate of overlapping points. All points are counted only once, hence leading to an underestimation of photoevents after the saturation point is reached.

FIGURE 4. Plot of Image Area above Threshold versus Signal Integration Time



V. CONCLUSIONS

During this second year, final system evaluations of The Hamamatsu C1966-20 VIM Photonic Microscopy System and the Gould IP8400 Programmable Image Display Unit were carried out. Upon receiving official approval, purchase of the items was ordered.

A Microstructural Analysis Routine (MSAR) was developed to analyze the extremely photon-limited events recorded by the System under development. A program (Particlet) obtained from the United States Army Medical Research Institute of Chemical Defense (USAMRICD) was used as a fundamental concept in successfully accomplishing this task.

Two peer-reviewed scientific manuscripts reporting upon significant aspects of the work to date were prepared and presented, accepted for publication, or published during this reporting period:

"Detection and Localization of Quantum-Limited Events from Radionuclide Labeled Material by Computer Enhanced Video Microscopy." RK Traub, NJ Pressman, JK Frost, PK Gupta, RL Showers, GW Gill, DL Cook and JK Frost, Jr. In the Optical Society of America's Topical Meeting on Quantum Limited Imaging and Image Processing Technical Digest, 86(6):63-66, 1986.

"A Quantitative Method for the Detection and Localization of Quantum-Limited Events From Radionuclides in Cells and Tissue Sections by Computer Enhanced Video Microscopy." NJ Pressman, JK Frost, PK Gupta, RL Showers, GW Gill, DL Cook, JK Frost, Jr and RK Traub. Presented at the International Academy of Cytology's Fifth International Conference on Automation of Diagnostic Cytology and Histology, Brussels, Belgium, May 30-June 1, 1986. Analyt Quant Cytol Histol, (accepted for publication).

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GLOSSARY

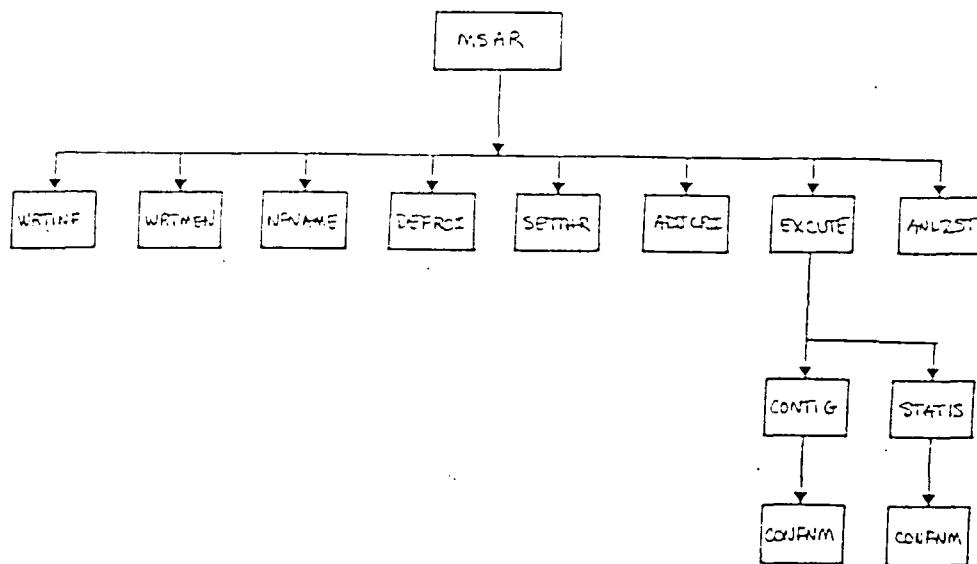
OF SELECTED KEY TERMS, UNITS, AND ABBREVIATIONS

AChE	-Acetylcholinesterase
attogram	- 10^{-18} gram
ASA	- American Standards Association, speed of photographic film
BIDAS	- The Johns Hopkins Biomedical Image Data Analysis System
Ci	- Curie = 3.7×10^{10} disintegrations per second
¹⁴ C	- Radioactive Carbon isotope with atomic mass of 14
C1966-20	- Hamamatsu VIM image processing system
CFIAC	- Cytotechnologist Fellow, International Academy of Cytology
cpm	- Counts per minute
cps	- Counts per second
DAGE-MTI	- Dage-MTI, Inc., Michigan City, IN 46360
DAMD17-84-C-4222	- USAMRICD Contract for "The Development of a System for Enhanced Radionuclide Detectors" with JHU
DEC	- Digital Equipment Corporation, Northboro, MA 01532
dps	- Disintegrations per second
EM	- Electron Microscopy
femtogram	- 10^{-15} gram
feret diameter	- The maximum diameter of a structure in a given direction
FIAC	- Fellow, International Academy of Cytology
FITC	- Fluorescein Isothiocyanate
flux	- Rate of transfer of particles or energy per unit area
GOULD	- Gould Inc., DeAnza Imaging & Graphics Division, San Jose, CA 95131
\hbar	- Planck's constant = 6.62363×10^{-34} joule-second
³ H	- Isotope of Hydrogen with atomic mass of 3
IAC	- International Academy of Cytology
IOD	- Integrated Optical Density
JHMI	- The Johns Hopkins Medical Institutions
JHU	- The Johns Hopkins University
mCi	- Millicurie = 10^{-3} curie
MCP	- Microchannel Plate

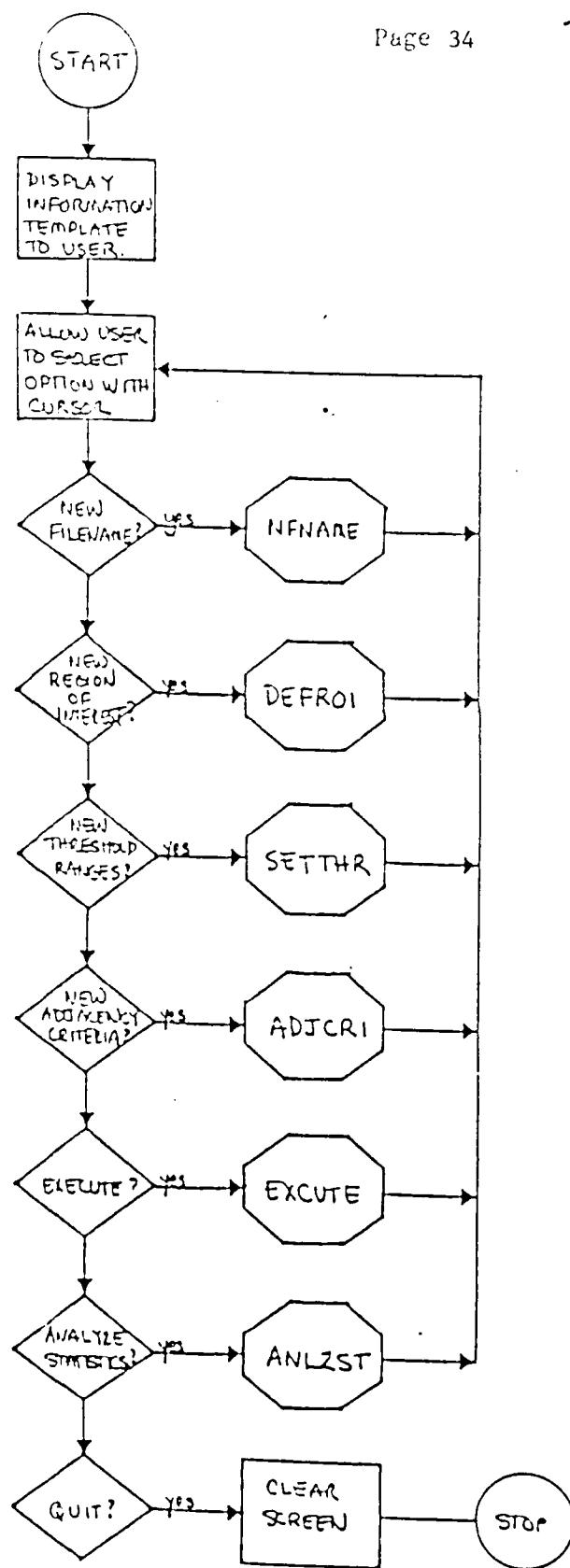
MIAC	- Member, International Academy of Cytology
MSAR	- The Microstructural Analysis Routine
OD _{max}	- Maximum Optical Density
OSA	- Optical Society of America
Particlet	- Particle counting and sizing program developed by USAMRICD
P ² /A	- Perimeter squared divided by Area
PI	- Principal Investigator
pixel	- Picture element
PMI	- Photonic Microscopy, Inc., Oak Brook, IL 60521
PMIAC	- Professional Member, International Academy of Cytology
RFP	- Request for Proposals
RGB	- Red/Green/Blue
ROI	- Region of Interest
slice-value	- Boundary value for a threshold range
specific activity	- Activity (i.e., disintegrations per second) in (micro-, milli-) curies of radioactive species per unit mass or concentration of non-radioactive material (e.g., 56 mc C ¹⁴ /mM experimental material)
S/N	- Signal to Noise ratio
³⁵ S	- Isotope of Sulfur with atomic mass of 35
USAMRICD	- U. S. Army Medical Research Institute for Chemical Defense
USAMRDC	- U. S. Army Medical Research and Development Command
VAX-11/780	- DEC 32-bit computer
VENUS	- Zeiss low-light-level video camera
VIM	- Video Intensified Microscopy
ZEISS	- Carl Zeiss, Inc., Thornwood, New York 10594

APPENDICES

Appendix A. MSAR Flowcharts

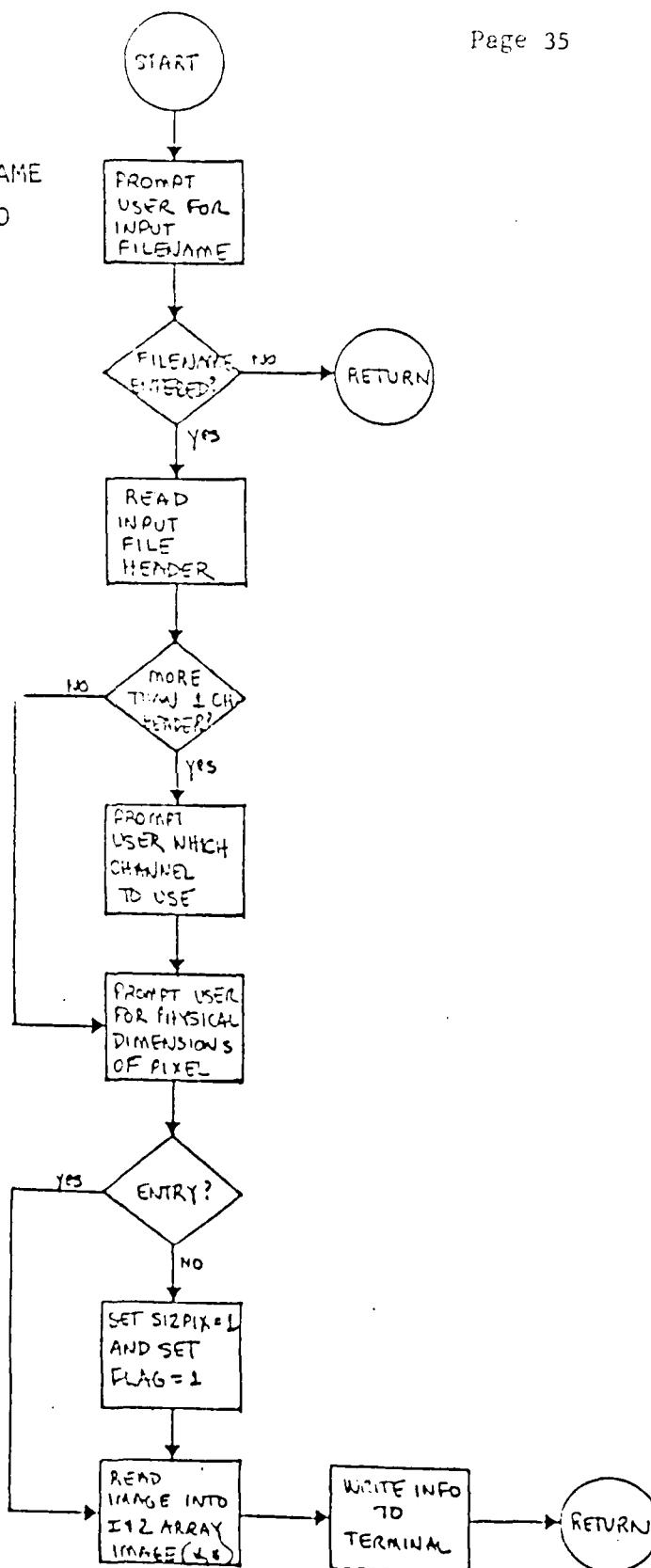


MAIN PROGRAM: EDC_MSAR



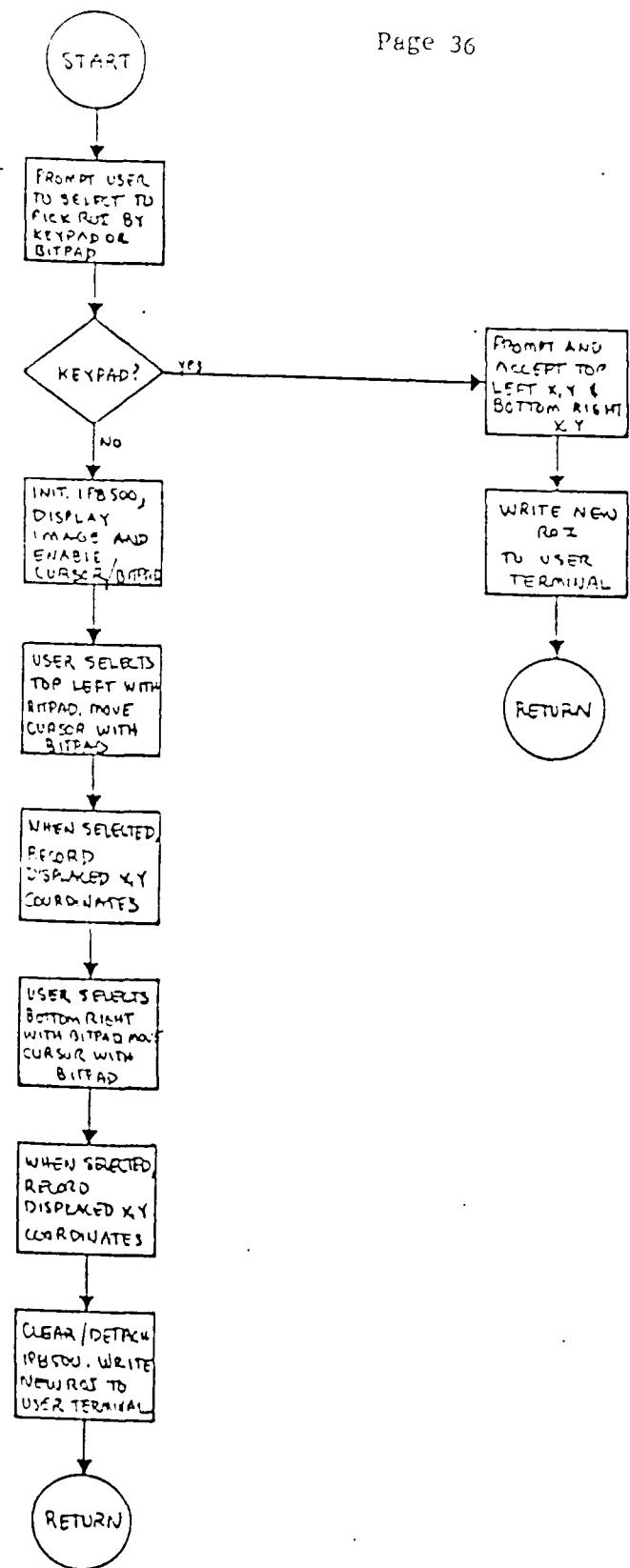
SUBROUTINE: NFNAME

-SUPPLY IMAGE FILENAME
AND READ IMAGE INTO
VAX MEMORY.



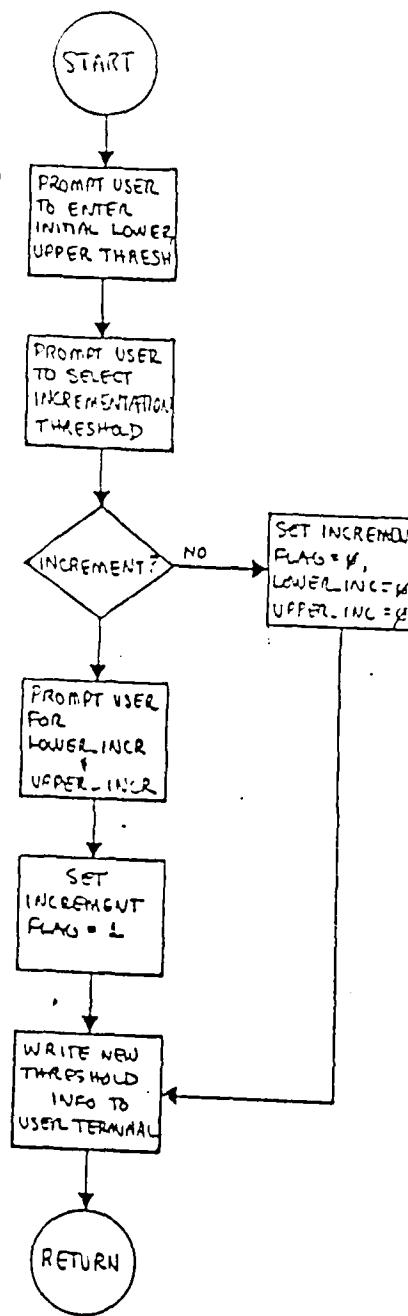
SUBROUTINE: DEFROI

-ALTER REGION OF INTEREST (ROI) OF IMAGE THAT THE CALCULATIONS ARE PERFORMED WITHIN.



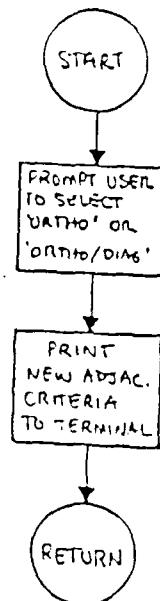
SUBROUTINE: SETTHR

-ALTER THRESHOLD VALUES,
THESE DETERMINE RANGE
OF PIXEL VALUES
CONSIDERED IN ANALYSIS.



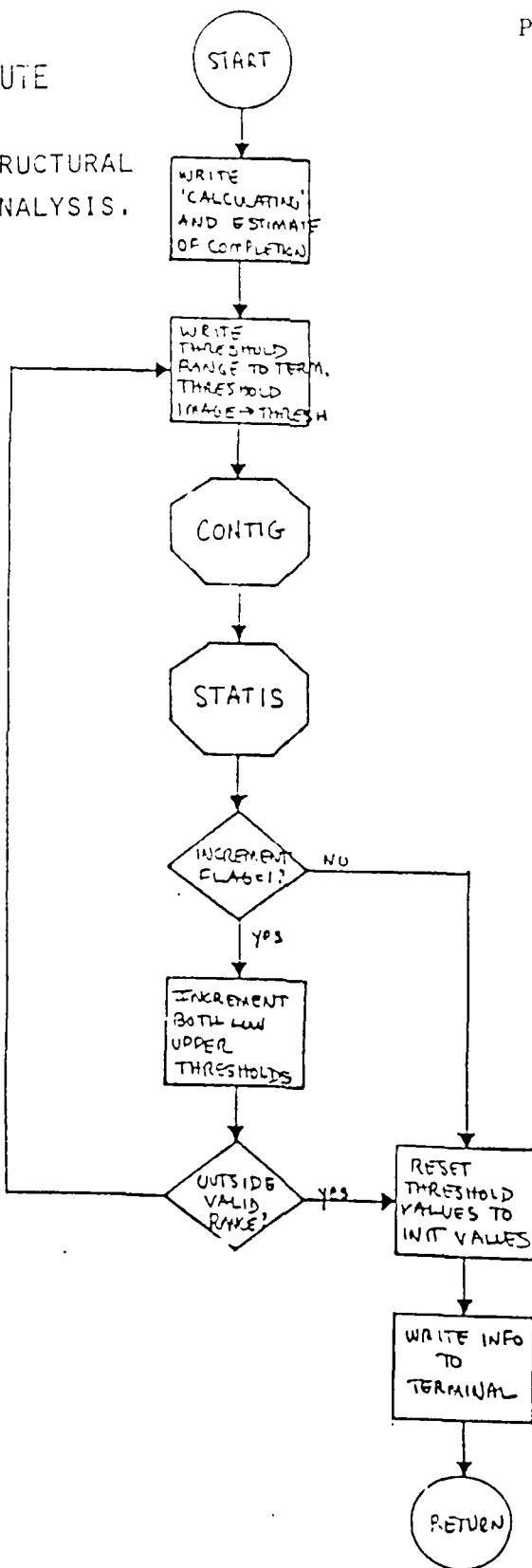
SUBROUTINE: ADJCRI

-ALTER RULE GOVERNING
CRITERIA FOR DETERMINING
ADJACENCY OF
NEIGHBORING PIXELS.



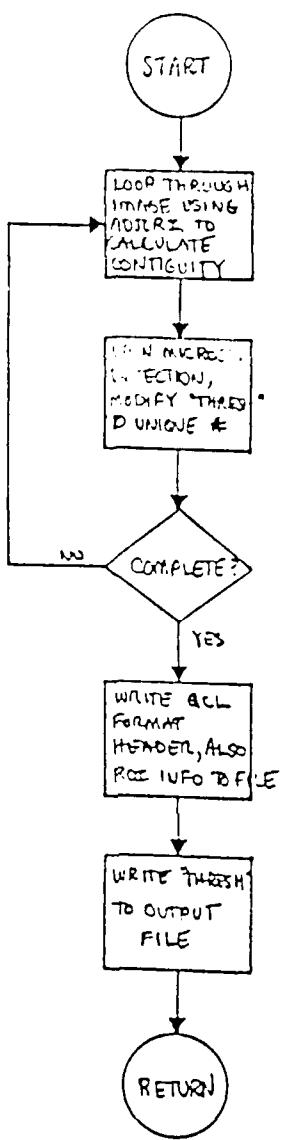
SUBROUTINE: EXECUTE

-PERFORM MICROSTRUCTURAL
DETECTION AND ANALYSIS.



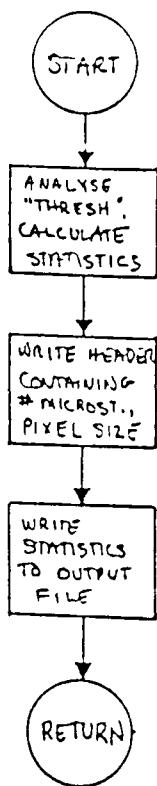
SUBROUTINE: CONTIG

-DETERMINE ADJACENCY
OF PIXELS AND
ASSIGN PIXELS TO
DISTINCT
MICROSTRUCTURES.



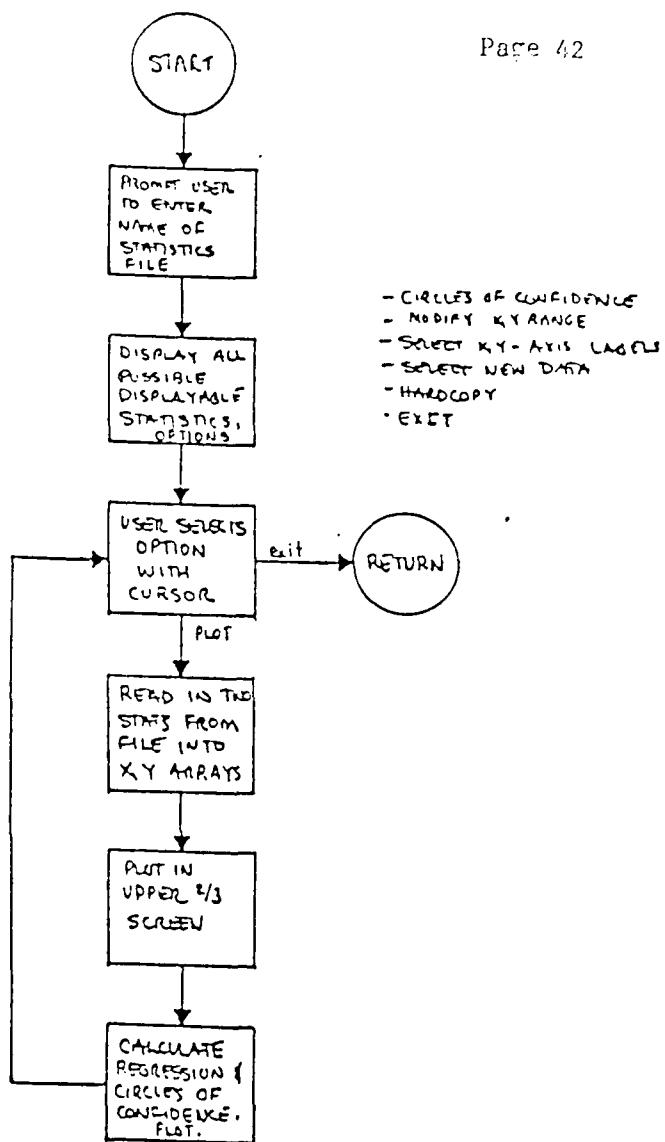
SUBROUTINE: STATIS

-CALCULATE STATISTICS
ON EACH
MICROSTRUCTURE.



SUBROUTINE: ANLZST

-MICROSTRUCTURAL
DATA ANALYSIS.



Appendix B. MSAR Program Code

```

PROGRAM MSAR
C... Read in a REAL *4 image from VAX, measure microstructure
C... information (e.g. number, geometric X&Y center, weighted X&Y center,
C... area, perimeter length, perimeter count, maximum horizontal and
C... vertical feret diameters, Integrated Optical Density and average
C... Optical Density.
C
INCLUDE 'EDC_MSARCOM.FOR'
BYTE INFTXT(80)
DATA IOPT/1/
C
C... PRINT RUNNING INFO TO USER
C
OPEN(UNIT=1,NAME='[EDC.TASKS]EDC_MSAR_TEXT.FOR',TYPE='OLD',
CREADONLY)
5   READ(1,10,END=15),NCHAR,(INFTXT(J),J=1,NCHAR)
10  FORMAT(8,<NCHAR>A1)
11  TYPE 12,(INFTXT(J),J=1,NCHAR)
12  FORMAT(' ',<NCHAR>A1)
GOTO 5
15  CLOSE(UNIT=1,DISPOSE='SAVE')
TYPE 16
16  FORMAT('$TYPE CARRIAGE RETURN TO CONTINUE : ')
ACCEPT 17,INFTXT(1)
17  FORMAT(A1)
C
C... DISPLAY INFO TO USER IN MENU SCREEN.
C
CALL WRTTMP
CALL WRTINF
C
C... DISPLAY MENU TO USER AT BOTTOM OF SCREEN
C
20  CALL WRTMEN
C
C... DISPLAY MENU SELECTION CURSOR. WAIT FOR USER TO SELECT OPTION
C
CALL SELOPT(IOPT)
GOTO (100,200,300,400,500,600),IOPT
GOTO 20
C
100 CALL FNNAME          !SET NEW FILENAME
GOTO 20
200 IF(FNMSET .EQ. 0) GOTO 900 !CHECK IF FILENAME NOT SPECIFIED
CALL DEFROI           !DEFINE ROI
GOTO 20
300 CALL ADJCRI          !ADJACENCY CRITERIA
GOTO 20
400 CALL SETTHR          !SET THRESHOLDS
GOTO 20
500 IF(FNMSET .EQ. 0) GOTO 900 !CHECK IF FILENAME NOT SPECIFIED
CALL EXECUTE          !EXECUTE
GOTO 20
600 CALL CLRSCR          !EXIT FROM ENTIRE PROGRAM
CALL CLRTXT
STOP
C
C... OPERATION CANNOT BE PERFORMED UNLESS FILE HAS BEEN READ INTO MEMORY

```

```
C  
900  CALL CLRIN(19,23)           !CLEAR LINES 19-23 (MENU LOCATION)  
      CALL SCROLL(19,23)          !SET SCROLL REGION FOR 19-23  
      TYPE 910  
910  FORMAT(/' OPERATION CANNOT BE PERFORMED UNTIL FILE HAS BEEN  
C READ INTO MEMORY')  
      T=SECNDS(0.)  
      CONTINUE  
      IF(SECNDS(0.)-T .LT. 2.0) GOTO 920  
      CALL SCROLL(1,24)  
      CALL CLRIN(19,23)  
      GOTO 20  
C  
      END  
C  
C  
C  
C  
C
```

```
SUBROUTINE WRITMP
C
C... WRITE TEMPLATE TO TERMINAL
C
CALL CLRTXT
CALL VTCURP(4,1)
TYPE 100
TYPE 120
TYPE 130
TYPE 140
TYPE 150
TYPE 160
TYPE 170
TYPE 180
TYPE 185
TYPE 190
TYPE 200
100 FORMAT('+FILENAME: //')
120 FORMAT(' ')
130 FORMAT(' THRESHOLD RANGE' ROI')
140 FORMAT(' LOWER:           UPPER:           ROWS:
C           COLUMNS: ')
150 FORMAT(' THRESHOLD INCREMENT:           X-LEFT:
C           Y-TOP: ')
160 FORMAT(' LOWER_INCR:           UPPER_INCR:           X-RIGHT:
C           Y-BOTTOM: ')
170 FORMAT(' ')
180 FORMAT(' ADJACENCY CRITERIA: ')
185 FORMAT(' PIXEL DIMENSION:           MICRONS')
190 FORMAT(' ')
200 FORMAT(' PROGRAM STATUS: ')
C
RETURN
END
C
C
C
C
```

```
SUBROUTINE WRTMEN
C
C... WRITE MENU TO TERMINAL
C
CHARACTER*10 OPTION(6)
INTEGER #2 OPTLEN(6),OPTLIN(6),OPTCOL(6)
BYTE BOLD(4), ATTOFF(4), REVERS(4)
DATA OPTION(1) // 'FILENAME' /           !MENU OPTIONS
DATA OPTION(2) // 'DEFINE ROI' /
DATA OPTION(3) // 'ADJACENCY' /
DATA OPTION(4) // 'THRESHOLD' /
DATA OPTION(5) // 'EXECUTE' /
DATA OPTION(6) // 'EXIT' /
DATA OPTLEN/8, 10, 9, 9, 7, 4/           !LENGTH OF MENU OPTIONS
DATA OPTLIN/20, 20, 20, 22, 22, 22/
DATA OPTCOL/1, 20, 39, 1, 20, 39/
DATA BOLD/27, 91, 49, 109/               !ESC [ 1 m
DATA ATTOFF/27, 91, 48, 109/              !ESC [ 0 m
DATA REVERS/27, 91, 55, 109/              !ESC [ 7 m
C
TYPE 90, (BOLD(J), J=1, 4)
90 FORMAT('*'4A1)
DO 100 IOPT=1, 6
CALL VTCURP(OPTLIN(IOPT), OPTCOL(IOPT))
TYPE 95, OPTION(IOPT)(1:10)
95 FORMAT('*'A10)
100 CONTINUE
RETURN
END
C
C
C
C
C
```

```

SUBROUTINE SELOPT(IOPT)
CHARACTER*10 OPTION(6)
INTEGER *2 OPTLEN(6), OPTLIN(6), OPTCOL(6)
BYTE BOLD(4), ATTOFF(4), REVERS(4), BLINK(4)
DATA OPTION(1) // 'FILENAME' !MENU OPTIONS
DATA OPTION(2) // 'DEFINE ROI'
DATA OPTION(3) // 'ADJACENCY'
DATA OPTION(4) // 'THRESHOLD'
DATA OPTION(5) // 'EXECUTE'
DATA OPTION(6) // 'EXIT'
DATA OPTLEN/8, 10, 9, 7, 4/ !LENGTH OF MENU OPTIONS
DATA OPTLIN/20, 20, 20, 22, 22, 22/
DATA OPTCOL/1, 20, 39, 1, 20, 39/
DATA BOLD/27, 91, 49, 109/ !ESC [ 1 m
DATA ATTOFF/27, 91, 48, 109/ !ESC [ 0 m
DATA REVERS/27, 91, 55, 109/ !ESC [ 7 m
DATA BLINK/27, 91, 53, 109/ !ESC [ 5 m
C
C      JOPT=IOPT
C
60   TYPE 90, (ATTOFF(J), J=1, 4)      !RETURN 'OLD' OPTION TO NORMAL
TYPE 90, (BOLD(J), J=1, 4)      !HOWEVER, BOLD THE OPTION
CALL VTCURP(OPTLIN(JOPT), OPTCOL(JOPT))
TYPE 95, OPTION(JOPT)(1:10)
C
C      TYPE 90, (REVERS(J), J=1, 4)      !'REVERSE' 'NEW' OPTION
C      TYPE 90, (BLINK(J), J=1, 4)      !'BLINK' 'NEW' OPTION
90   FORMAT('+'A1)
CALL VTCURP(OPTLIN(IOPT), OPTCOL(IOPT))
TYPE 95, OPTION(IOPT)(1:10)
95   FORMAT('+'A10)
CALL VTCURP(23, 1)
C
C...  WAIT FOR USER TO MOVE MENU CURSOR
C
IKEY=0
TLAST=SECNDS(0.)           !SECONDS SINCE LAST INPUT
100  I=IESC(IKEY)
IF(IKEY .EQ. 0) THEN
    IF(SECNDS(0.)-TLAST .GT. 3600.) THEN
        IOPT=6
        GOTO 999
    END IF
    GOTO 100
END IF
IF(IKEY .EQ. 13) GOTO 999      !<CR> USER ACCEPTS IOPT
IF(IKEY .LT. 65 .OR. IKEY .GT. 68) GOTO 100
JOPT=IOPT
IF(IKEY .EQ. 65) THEN          !UP ARROW
    IOPT=IOPT-3
    IF(IOPT .LT. 1) IOPT=IOPT+6
END IF
IF(IKEY .EQ. 66) THEN          !DOWN ARROW
    IOPT=IOPT+3
    IF(IOPT .GT. 6) IOPT=IOPT-6
END IF
IF(IKEY .EQ. 67) THEN          !RIGHT ARROW
    IOPT=IOPT+1
    IF(IOPT .GT. 6) IOPT=1
END IF

```

```
IF(IKEY .EQ. 68) THEN           !LEFT ARROW
    IOPT=IOPT-1
    IF(IOPT .LT. 1) IOPT=6
END IF
GOTO 60
C
999   TYPE 90,(ATTOFF(J),J=1,4)      !RETURN OPTION TO NORMAL
      RETURN
END
C
C
C
C
C
```

```

SUBROUTINE NFNAME
INCLUDE 'EDC_MSARCDM.FOR'

C
C... SETUP SOME PARAMETERS
C
IRROW=512
IRCOL=512
IRXTOP=1
IRYTOP=1
IRXBOT=512
IRYBOT=512

C
C... CLEAR MENU SECTION OF VT SCREEN
C
CALL CLRLIN(19,23)           !CLEAR LINES 19-23 (MENU LOCATION)
CALL SCROLL(19,23)           !SET SCROLL REGION FOR 19-23

C
C... PROMPT USER FOR FILENAME
C
DO 90 J=1,14
90  FNAME(J)=0
TYPE 100
100 FORMAT('ENTER FILENAME AND EXTENSION : ')
ACCEPT 120,NCHAR,(FNAME(J),J=1,NCHAR)
120 FORMAT(Q,13A1)
IF(NCHAR .EQ. 0) THEN
    CALL SCROLL(1,24)
    CALL CLRLIN(19,23)
    GOTO 999
END IF

C
C... GET PIXEL DIMENSIONS FROM USER
C
300  TYPE 320
320  FORMAT(' ENTER PHYSICAL DIMENSION OF PIXEL IN MICRONS
C <ie 0.25>')
TYPE 330
330  FORMAT('$TYPE <CR> IF UNKNOWN : ')
ACCEPT 340,NCHAR,SIZPIX
340  FORMAT(Q,F15.5)
IPIXFG=0                      !SET PIXEL FLAG=0
IF(NCHAR .EQ. 0) THEN
    IPIXFG=1                  !SET PIXEL FLAG SINCE USER DIDN'T KNOW SIZE
    SIZPIX=1.0
END IF

C
C... OPEN INPUT FILE
C
OPEN(UNIT=3,NAME=FNAME,TYPE='OLD',READONLY,FORM='UNFORMATTED',
CERR=900)

C
C... READ IMAGE INTO MEMORY
C
TYPE 350
350  FORMAT(////' READING IMAGE FILE INTO MEMORY'//)
READ (3) TEMP
CLOSE(UNIT=3,DISPOSE='SAVE')
NCOLS=512
NROWS=512
DO 400 J=1,512                !TRANSFER TO I*2 ARRAY

```

```
        DO 400 K=1,512
400      IMAGE(K,J)=IINT(TEMP(K,J))
C
C...   WRITE NEW INFORMATION TO TERMINAL
C
        CALL SCROLL(1,24)
        CALL WRTINF          !WRITE INFO TO SCREEN
        CALL CLRIN(19,23)
        FNMSET=1              !SET FLAG TO INDICATE FILE READ SUCCESSFULLY
C
999      RETURN
C
C
900      TYPE 910
910      FORMAT(' ERROR IN OPENING FILE. PLEASE REENTER')
        GOTO 80
        END
C
C
C
C
```

```
SUBROUTINE DEFROI
INCLUDE 'EDC_MSARCOM.FOR'

C
C... CLEAR MENU SECTION OF VT SCREEN
C
CALL CLRIN(1B,23)           !CLEAR LINES 1B-23 (MENU LOCATION)
CALL SCROLL(1B,23)          !SET SCROLL REGION FOR 1B-23

C
C... KEYPAD ENTRY
C
200 TYPE 220
220 FORMAT('ENTER X,Y COORDINATES OF TOP LEFT CORNER OF ROI : ')
ACCEPT 240,NCHAR,I1,I2
240 FORMAT(0,2I4)
IF(NCHAR.EQ.0) GOTO 999
IF(I1.LT.0.OR.I2.LT.0) GOTO 300
IF(I1.GT.NCOLS.OR.I2.GT.NROWS) GOTO 300

C
250 TYPE 260
260 FORMAT('ENTER X,Y COORDINATES OF BOTTOM RIGHT CORNER OF ROI : ')
ACCEPT 240,NCHAR,I3,I4
IF(NCHAR.EQ.0) GOTO 999
IF(I3.LT.I1.OR.I4.LT.I2) GOTO 320
IF(I3.GT.NCOLS.OR.I4.GT.NROWS) GOTO 300

C
IRXTOP=I1
IRYTOP=I2
IRXBOT=I3
IRYBOT=I4
IRROW=IRYBOT-IRYTOP+1
IRCOL=IRXBOT-IRXTOP+1
GOTO 999

C
C... ERRORS IN ENTERING ROI INFORMATION
C
300 TYPE 310
310 FORMAT(' BOUNDARY DEFINITION ARE OUTSIDE OF LEGAL RANGE')
GOTO 200
320 TYPE 330
330 FORMAT(' BOTTOM RIGHT MUST BE BELOW AND TO THE RIGHT OF TOP LEFT')
GOTO 250

C
999 CALL SCROLL(1,24)
CALL WRTINF      !WRITE INFO TO SCREEN
CALL CLRIN(1B,23)

C
RETURN
END

C
C
C
C
C
```

```

SUBROUTINE SETTHR
INCLUDE 'EDC_MSARCOM.FDR'
BYTE ACCEPT
C
C... CLEAR MENU SECTION OF VT SCREEN
C
C     CALL CLRLLIN(19,23)           !CLEAR LINES 19-23 (MENU LOCATION)
C     CALL SCROLL(19,23)           !SET SCROLL REGION FOR 19-23
C
C... GET THRESHOLD VALUES FROM USER
C
90   TYPE 100
100  FORMAT('ENTER INITIAL LOWER,UPPER THRESHOLD VALUES : ')
    ACCEPT 120,NCHAR,I1,I2
120  FORMAT(0.214)
    IF(NCHAR .EQ. 0) GOTO 999
    IF(I1 .LT. 0 .OR. I2 .LT. I1) GOTO 800
    IF(I2 .GT. 255 .OR. I1 .GT. I2) GOTO 800
C
C     TYPE 160
160  FORMAT('ENABLE THRESHOLD INCREMENTATION ? (N/Y) : ')
    ACCEPT 170,NCHAR,ACCEPT
170  FORMAT(0,A1)
    IF(ACCEPT .EQ. 'Y') GOTO 190
C
C... USER DID NOT WANT THRESHOLD INCREMENTATION. SET VALUES AND RETURN
C
C     ITLOW=I1
C     ITUPR=I2
C     ITINCR=0
C     ITLINC=0
C     ITUINC=0
    GOTO 999
C
C... GET INCREMENTATION VALUES FROM USER
C
190  TYPE 200
200  FORMAT('ENTER LOWER,UPPER THRESHOLD INCREMENT VALUES : ')
    ACCEPT 120,NCHAR,I3,I4
    IF(NCHAR .EQ. 0) GOTO 999
    IF(I3 .GT. 255 .OR. I4 .GT. 255) GOTO 820
    IF(I3 .EQ. 0 .AND. I4 .EQ. 0) GOTO 840
C
C... SET VALUES AND RETURN
C
C     ITLOW=I1
C     ITUPR=I2
C     ITINCR=1
C     ITLINC=I3
C     ITUINC=I4
    GOTO 999
C
800  TYPE 810
810  FORMAT(// ' INVALID THRESHOLD VALUES. PLEASE RE-ENTER'//)
    GOTO 90
C
820  TYPE 830
830  FORMAT(// ' THRESHOLD INCREMENT VALUE CANNOT EXCEED 255. PLEASE
    C RE-ENTER'//)
    GOTO 190

```

```
C  
B40    TYPE B50  
B50    FORMAT(//, INVALID INCREMENT VALUES. PLEASE RE-ENTER//)  
      GOTO 190  
C  
999    CALL SCROLL(1,24)          !WRITE INFO TO SCREEN  
      CALL WRITINF  
      CALL CLRIN(18,23)  
C  
      RETURN  
      END  
C  
C  
C  
C  
C
```

```
SUBROUTINE ADJCRI
INCLUDE 'EDC_MSARCOM.FOR'
C
C...  CLEAR MENU SECTION OF VT SCREEN
C
C      CALL CLRIN(19,23)          !CLEAR LINES 19-23 (MENU LOCATION)
C      CALL SCROLL(19,23)         !SET SCROLL REGION FOR 19-23
C
C...  GET USER INPUT AND CHANGE ADJACENCY CRITERIA
C
90   TYPE 100
100  FORMAT('' SELECT CRITERIA BY WHICH POINTS ARE CONSIDERED
C CONTIGUOUS'' 1-ORTHOGONAL POINTS ONLY'' 2-ORTHOGONAL AND
C DIAGONAL POINTS')
      TYPE 120
120  FORMAT('*ENTER SELECTION : ')
      ACCEPT 140,NCHAR,ISEL
140  FORMAT(0,12)
      IF(NCHAR .EQ. 0) GOTO 999
      IF(ISEL .LT. 1 .OR. ISEL .GT. 2) GOTO 90
      IF(ISEL .EQ. 1) IADJCT=0
      IF(ISEL .EQ. 2) IADJCT=1
C
999  CALL SCROLL(1,24)           !WRITE INFO TO SCREEN
      CALL WRITINF
      CALL CLRIN(19,23)
C
C...  RETURN
END
C
C
C
C
C
```

```

SUBROUTINE EXECUTE
INCLUDE 'EDC_MSARCOM.FOR'

C
C... SAVE INITIAL THRESHOLD VALUES
C
JTLLOW=ITLOW
JTUPR=ITUPR
NUMAVG=0

C
C... CHANGE PROGRAM STATUS TO 'EXECUTING'
C
STATUS=2
CALL WRTINF

C
C... OPEN OUTPUT FILE TO CONTAIN 'AVERAGE' INFORMATION OF MICROSTRUCTURES
C
OPEN(UNIT=2,NAME='MICROS.DAT',TYPE='UNKNOWN',ACCESS='APPEND')
DO 10 J=1,14
IF(FNAME(J) .EQ. '.') GOTO 15
CONTINUE
NCHAR=14
GOTO 20
15 NCHAR=J+3
20 WRITE(2,25), (FNAME(J),J=1,NCHAR),IRXTOP,IRYTOP,IRXBOT,IRYBOT
25 FORMAT(//> FILENAME = '<NCHAR>A1//> REGION OF INTEREST - '
CTOP LEFT (X='I3',Y='I3'), BOTTOM RIGHT (X='I3',Y='I3')//>
      WRITE(2,30)                                     !PUT OUT HEADER OF FIRST SECTION
30 FORMAT('
          AVERAGE      AVERAGE      AVERAGE'
          '           NUMBER      WEIGHTED      WEIGHTED'
          '           TOTAL       OBJECTS      OBJECT
          '           X-         Y-         X-         Y-'
          '           RANGE      AREA       (MICROSTRUCTURES)      AREA
          '           CENTER     CENTER     CENTER      CENTER')
C
C
C... CLEAR MENU SECTION OF VT SCREEN
C
CALL CLRIN(19,23)           !CLEAR LINES 19-23 (MENU LOCATION)
CALL SCROLL(19,23)          !SET SCROLL REGION FOR 19-23

C
C... GO THROUGH THE INPUT IMAGE FILE IN ARRAY 'IMAGE', THRESHOLD USING
C... THE USER-SUPPLIED VALUES, AND PLACE THE BINARY RESULT IN 'THRESH'.
C... ONLY BE CONCERNED WITH THE REGION OF INTEREST.
C
50 DO 100 I=IRYTOP,IRYBOT
TYPE 99.1
99 FORMAT(' THRESHING LOC 'I3)
DO 100 J=IRXTOP,IRXBOT
IF(IMAGE(J,I) .GE. ITLOW .AND. IMAGE(J,I) .LE. ITUPR) THEN
  THRESH(J,I)=1
C... *** NOTE - NEXT LINE OF CODE IS FOR VERTICAL FILL OF IMAGE.
  IF(IMAGE(J,I-1) .LT. ITLOW .OR. IMAGE(J,I-1)
C.GT. ITUPR) THRESH(J,I-1)=1
  ELSE
    THRESH(J,I)=0
  END IF
  CONTINUE
100 C

```

```

C... GO BACK INTO IMAGE AND SEARCH FOR -1 FLAG. IF FOUND, PUT THE VALUE
C... OF THE PIXEL DIRECTLY VERTICAL TO THE PRESENT PIXEL INTO PRESENT
C... LOCATION.
C
C DO 2100 I=IRYTOP,IRYBOT
C TYPE 99,I
C DO 2100 J=IRXTOP,IRXBOT
C IF(THRESH(J,I) .EQ. -1) THEN
C     THRESH(J,I)=1
C     IMAGE(J,I)=IMAGE(J,I+1)
C END IF
2100 CONTINUE
C
C... CALL SUBROUTINE 'CONTIG' TO CALCULATE THE CONTIGUOUS STRUCTURES
C... IN THE USER-DEFINED ROI AND USING THE USER-SPECIFIED ADJACENCY
C... CRITERIA. RESULT OF THIS SUBROUTINE WILL BE UNIQUELY LABEL
C... MICROSTRUCTURES IN THE ARRAY 'THRESH'.
C
C CALL CONTIG
C TYPE 101,UNIQUE
101 FORMAT(' UNIQUE NUMBER = 'I7)
T=SECNDS(0.)
102 CONTINUE
IF(SECNDS(0.)-T .LT. 2.0) GOTO 102
C
C... CALL SUBROUTINE 'STATIS' TO CALCULATE THE STATISTICS ON EACH
C... OF THE UNIQUE MICROSTRUCTURES IN THE ARRAY 'THRESH'. WRITE
C... OUTPUT TO OUTPUT FILE
C
C CALL STATIS(NUMAVG)
C
C... WAS THRESHOLDING INCREMENTATION ENABLED?
C
C IF(ITINCR .EQ. 0) GOTO 900
C
C... INCREMENT THRESHOLDS
C
ITLOW=ITLOW+ITLINC
ITUPR=ITUPR+ITUINC
IF(ITLOW .LT. 0 .OR. ITLOW .GT. 255) GOTO 900 !OUTSIDE VALID RANGE
IF(ITUPR .LT. 0 .OR. ITUPR .GT. 255) GOTO 900
IF(ITLOW .GT. ITUPR) GOTO 900
C
C... WRITE INFORMATION TO TERMINAL
C
CALL SCROLL(1,24)
CALL WRTINF
CALL CLR琳(19,23)           !CLEAR LINES 19-23 (MENU LOCATION)
CALL SCROLL(19,23)           !SET SCROLL REGION FOR 19-23
GOTO 50
C
C... RESET INITIAL THRESHOLD AND THEN RETURN
C
900 ITLOW=JTLOW
ITUPR=JTUPR
C
C... PUT A <FF> IN FILE TO SEPERATE THIS RUN
C
910 WRITE(2,910),12
FORMAT(' ',A1)

```

```
C...      WRITE SECOND PAGE OF INFO
        WRITE(2,915)
915      FORMAT('          AVERAGE          AVERAGE'
C'          AVERAGE          AVERAGE'
C'          TOTAL          PERIMETER          A
CVERAGE      HORIZ      VERTICAL          AVERAGE
C'          AVERAGE'
C'          THRESHOLD      PERIMETER      POINTS      TOTAL      PERI
CMETER      FERET      FERET      CUMULATIVE      IOD
C'          DDmax'
C'          RANGE          POINTS          PER OBJECT      PERIMETER      PER O
CBOJECT      DIAMETER      DIAMETER      IOD.          PER OBJECT
C'          PER OBJECT')
DO 930 J=1,NUMAVG
        WRITE(2,920),IAVG(J,1),IAVG(J,2),IAVG(J,3),GAVG(J,1),GAVG(J,7),
CGAVG(J,2),GAVG(J,3),GAVG(J,4),IAVG(J,4),GAVG(J,5),GAVG(J,6)
920      FORMAT(' ',I3,'-',I3,6X,I6,5X,F10.2,4X,F10.2,6X,F7.2,6X,F7.2,
C5X,F7.2,5X,I10,3X,F12.2,4X,F7.2)
930      CONTINUE
        WRITE(2,910),12
        CLOSE(UNIT=2,DISPOSE='SAVE')      !CLOSE 'AVERAGE' FILE
C
999      CALL SCROLL(1,24)
        STATUS=1                  !RESET STATUS TO USER-INPUT
        CALL WRTINF                !WRITE INFO TO SCREEN
        CALL CLRIN(18,23)
C
        RETURN
        END
C
C
C
C
C
```

```

SUBROUTINE CONTIG
INCLUDE 'EDC_MSARCOM.FDR'
BYTE OUTFIL(22),LOW(3),UPR(3),EXT(3)
INTEGER*2 START(256),STOP(256),IADJPT(512),DROP(65536),MAP(65536)
DATA EXT/'M','C','S'/
C
C      UNIQUE=1
C      IDROP=0
C...
C...   LOOP THROUGH ALL LINES
C
DO 450 IROW=IRYTOP,IRYBOT
TYPE 451,IROW
451 FORMAT(' ROW = '17)
C
C...   SEARCH LINE FOR START AND STOP OF STRUCTURES ON LINE. THE ARRAYS
C...   START(*) AND STOP(*) CONTAIN THE STARTING AND STOPPING LOCATIONS
C...   OF ALL THE STRUCTURES ON THAT LINE. 'ISNUM' CONTAINS THE NUMBER OF
C...   STRUCTURES.
C
ISNUM=0
JCOL=IRXTOP
90 IF(THRESH(JCOL,IROW) .EQ. 0) GOTO 140
ISNUM=ISNUM+1
START(ISNUM)=JCOL
JCOL=JCOL+1
100 IF(JCOL .GT. IRXBOT) GOTO 110
IF(THRESH(JCOL,IROW) .EQ. 1) GOTO 100
110 STOP(ISNUM)=JCOL-1
140 JCOL=JCOL+1
IF(JCOL .LE. IRXBOT) GOTO 90
C
C...   SEARCH EACH STRUCTURE FOR CONTIGUOUS STRUCTURES ABOVE
C
DO 400 ISTRCT=1,ISNUM
IF(IROW .EQ. IRYTOP) GOTO 360           !TOP ROW
C
C...   LOOP THROUGH STRUCTURE. SEARCH FOR ADJACENT STRUCTURES. IF FOUND,
C...   MAKE NOTE OF UNIQUE NUMBER OF ADJACENT STRUCTURE.
C
IADJ=0
DO 300 JCOL=START(ISTRCT),STOP(ISTRCT)
IF(THRESH(JCOL,IROW-1) .EQ. 0) GOTO 200
C...
ADJACENT POINT FOUND
IF(IADJ .EQ. 0) GOTO 190
DO 180 J=1,IADJ
IF(THRESH(JCOL,IROW-1) .EQ. IADJPT(J)) GOTO 200 !UNIQUE # ALREADY FOUND
180 CONTINUE
190 IADJ=IADJ+1
IADJPT(IADJ)=THRESH(JCOL,IROW-1)
C
200 IF(IADJCT .EQ. 0) GOTO 300           !DONT CHECK FOR DIAGONALS
IF(JCOL-1 .LT. IRXTOP) GOTO 230        !DONT CHECK OUTSIDE ROI
IF(THRESH(JCOL-1,IROW-1) .EQ. 0) GOTO 230
C...
ADJACENT POINT FOUND
IF(IADJ .EQ. 0) GOTO 220
DO 210 J=1,IADJ
IF(THRESH(JCOL-1,IROW-1) .EQ. IADJPT(J)) GOTO 230           !UNIQUE # ALREADY FOUND
210 CONTINUE
220 IADJ=IADJ+1

```

```

IADJPT(IADJ)=THRESH(JCOL-1, IROW-1)
C
230 IF(JCOL+1 .GT. IRXBOT) GOTO 300      !DONT CHECK OUTSIDE ROI
IF(THRESH(JCOL+1, IROW-1) .EQ. 0) GOTO 300
C...
ADJACENT POINT FOUND
IF(IADJ .EQ. 0) GOTO 250
DO 240 J=1, IADJ
IF(THRESH(JCOL+1, IROW-1) .EQ. IADJPT(J)) GOTO 300      !UNIQUE # ALREADY FOUND
240 CONTINUE
250 IADJ=IADJ+1
IADJPT(IADJ)=THRESH(JCOL+1, IROW-1)
300 CONTINUE
C
IF(IADJ .EQ. 0) GOTO 360      !NO ADJACENT POINTS. ASSIGN NEXT VALUE
IF(IADJ .EQ. 1) GOTO 380      !ONE ADJACENT POINT
C
C... MORE THAN ONE STRUCTURE IS ADJACENT. SET PRESENT STRUCTURE TO LOWEST
C... ADJACENT STRUCTURE VALUE, THEN SEARCH FOR AND RESET ALL OTHER PREVIOUS
C... STRUCTURE VALUES TO THAT SAME LOW VALUE. SEARCH BY GOING FROM PREVIOUS
C... LINE TO THE FIRST LINE (BOTTOM TO TOP). WHEN STRUCTURE VALUE IS NO
C... LONGER ENCOUNTERED, DROP IT FROM THE SEARCH
C
IMIN=99999
DO 310 J=1, IADJ      !SELECT MINIMUM STRUCTURE VALUE
IF(IADJPT(J) .LT. IMIN) IMIN=IADJPT(J)
310 CONTINUE
C
DO 315 J=1, IADJ      !MAKE NOTE OF DROPPED VALUES
IF(IADJPT(J) .EQ. IMIN) GOTO 315
IDROP=IDROP+1
DROP(IDROP)=IADJPT(J)
315 CONTINUE
C
DO 320 JCOL=START(ISTRCT), STOP(ISTRCT)  !ASSIGN MIN VALUE TO 'THRESH'
THRESH(JCOL, IROW)=IMIN
320 CONTINUE
C
DO 350 K=1, IADJ
IF(IADJPT(K) .EQ. IMIN) GOTO 340      !DON'T DROP IMIN VALUE
DO 340 I=IROW-1, IRYTOP, -1
IFLG=0
DO 330 J=IRXTOP, IRXBOT
IF(THRESH(J, I) .NE. IADJPT(K)) GOTO 330
THRESH(J, I)=IMIN      !RESET VALUE
IFLG=1
330 CONTINUE
IF(IFLG .EQ. 0) GOTO 350      !DIDN'T FIND ON LINE. STOP SEARCH
340 CONTINUE
350 CONTINUE
GOTO 400
C
C... ASSIGN THE STRUCTURE NEXT UNIQUE VALUES. NO ADJACENT POINTS
C
360 DO 370 JCOL=START(ISTRCT), STOP(ISTRCT)
THRESH(JCOL, IROW)=UNIQUE
370 CONTINUE
UNIQUE=UNIQUE+1
GOTO 400
C
C... ASSIGN THE STRUCTURE THE VALUE OF THE SINGLE ADJACENT STRUCTURE

```

```

C
380    DD 390 JCOL=START(ISTRCT),STOP(ISTRCT)
        THRESH(JCOL, IROW)=IADJPT(1)
390    CONTINUE
        GOTO 400
C
400    CONTINUE
450    CONTINUE
C
C...   AT THIS POINT, THRESH CONTAINS UNIQUELY LABELLED MICROSTRUCTURES.
C...   HOWEVER, THE UNIQUE NUMBERS MUST BE COMPRESSED SINCE MANY VALUES
C...   WERE DROPPED.  THE VALUES DROPPED ARE IN THE ARRAY 'DROP(IDROP)'.
C...   THIS IS ACCOMPLISHED BY CREATED A 'MAP', COMPRESSING THE MAP AND
C...   THEN USING THAT COMPRESSED MAP TO ALTER THE VALUES IN 'THRESH'
C
        UNIQUE=UNIQUE-1
C
        TYPE 452,UNIQUE
452    FORMAT(' NUMBER OF MSTs BEFORE COMPRESSION = ',I10//':')
        TYPE 454,UNIQUE, IDROP
454    FORMAT(' UNIQUE = ',I7' IDROP = ',I7)
        ICTMAP=0
C
        MAP(0)=0                      !CLEAR MAP
        DO 600 J=1,UNIQUE
600    MAP(J)=0
C
        DO 610 JD=1, IDROP           !SETUP DROPPED POINTS
610    MAP(DROP(JD))=-1
C
        DO 620 J=1,UNIQUE           !FILL IN MAP
        IF(MAP(J) .EQ. -1) THEN
            MAP(J)=0
            GOTO 620
        END IF
        ICTMAP=ICTMAP+1
        MAP(J)=ICTMAP
620    CONTINUE
        UNIQUE=ICTMAP
C
        DO 640 IROW=IRYTOP,IRYBOT      !APPLY MAP TO 'THRESH'
        DO 640 JCOL=IRXTOP,IRXBOT
        IF(THRESH(JCOL, IROW) .EQ. 0) GOTO 640
        THRESH(JCOL, IROW)=MAP(THRESH(JCOL, IROW))
640    CONTINUE
C
C...   WRITE TO OUTPUT FILE
C
        CALL CONFM(OUTFIL,EXT)          !GET OUTPUT FILENAME
        OPEN(UNIT=3,NAME=OUTFIL,TYPE='NEW')
        NCHANS=1                        !ONE CHANNEL OF DATA
        NHEADR=2                        !NUMBER OF HEADER LINES
        WRITE(3,650),NROWS,NCOLS,NCHANS,NHEADR
650    FORMAT(4I4)
C...
        TYPE CHANNEL USED, ROI INFO, ADJACENCY FLAG AND THRESHOLD RANGE
        WRITE(3,670),ICHAN,IRXTOP,IRYTOP,IRXBOT,IRYBOT,IADJCT,ITLOW,ITUPR
670    FORMAT(B17)
C...
        WRITE 'THRESH' ARRAY TO FILE
        DO 685 I=IRYTOP,IRYBOT
        WRITE(3,680),(THRESH(J,I),J=IRXTOP,IRXBOT)

```

```
680      FORMAT(2216)
685      CONTINUE
          CLOSE(UNIT=3,DISPOSE='SAVE')
C
C      RETURN
END
C
C
C
C
```

```

SUBROUTINE STATIS(NUMAVG)
INCLUDE 'EDC_MSARCOM.FOR'
INTEGER*2 COORD(2,8),PERMCT,ORTHO,DIAG,ODmax,PERMC1,ICOUNT(8)
INTEGER ODmaxS
BYTE OUTFIL(22),EXT(3)
DATA COORD/-1,-1,-1,0,-1,1,0,1,1,1,1,0,1,-1,0,-1/
DATA EXT/'M','S','T'/
SQRTWO=2**0.5
C
C... OPEN FILE FOR MICROSTRUCTURE STATISTICS. WRITE HEADER INFO
C
CALL CONFNM(OUTFIL,EXT)           !GET OUTPUT FILENAME
OPEN(UNIT=3,NAME=OUTFIL,TYPE='NEW')
WRITE(3,10),OUTFIL,ITLOW,ITUPR
10 FORMAT(' FILENAME = ''22A1,T40' LOWER THRESHOLD = ''I3' UPPER
C THRESHOLD = ''I3)
WRITE(3,12),UNIQUE               !IMST,PIX SIZE, SIZE FLAG
12 FORMAT(' '15' UNIQUE MICROSTRUCTURES FOUND.')
IF(IPIXFC .NE. 1) THEN
    WRITE(3,14),SIZPIX,IPIXFG
14 FORMAT(' ACTUAL PIXEL SIZE = ''F7.3' MICRONS. FLAG = ''I2)
ELSE
    WRITE(3,16),SIZPIX,IPIXFG
16 FORMAT(' DEFAULT PIXEL SIZE = ''F7.3' MICRONS. FLAG = ''I2)
END IF
WRITE(3,22)
WRITE(3,24)
WRITE(3,26)
22 FORMAT(///'                                     WEIGHTED WEIGHTED
C                                     MAXIMUM      MAXIMUM')
24 FORMAT('     UNIQUE          X          Y          X          Y
C PERIMETER   PERIMETER   HORIZONTAL   VERTICAL
C           AVERAGE')
26 FORMAT(' MICROSTRUCTURE   CENTER   CENTER   CENTER   CENTER
C           AREA     COUNT    LENGTH   RUN-LENGTH  RUN-LENGTH  IOD
C           IOD     ODmax'')
C
C... CLEAR SOME VALUES
C
IAREAS=0
XCEN1=0
YCEN1=0
XWCEN1=0
YWCEN1=0
XTCENT=0
YTCENT=0
PERMC1=0
PERIM1=0.
IMAX_X_SUM=0
IMAX_Y_SUM=0
IODSUM=0
ODmaxS=0
C
C... CALCULATE STATISTICS FOR ALL MICROSTRUCTURES
C
DO 900 IMST=1,UNIQUE
C
C... SEARCH FOR FIRST OCCURANCE OF MICROSTRUCTURE #IMST
C
DO 100 IROW=IRYTOP,IRYBOT

```

```

DO 100 ICOL=IRXTOP, IRXBOT
IF(THRESH(ICOL, IROW) .EQ. IMST) GOTO 110
CONTINUE
C
C... CALCULATE PERIMETER BY SEARCHING ADJACENT POINTS. IF IADJCT=0,
C... SEARCH #2, 4, 6, 8. IF IADJCT=1, SEARCH #1, 2, 3, 4, 5, 6, 7, 8.
C...
C...      1 2 3
C...      8 * 4
C...      7 6 5
C
C... SINCE FIRST POINT WAS FOUND BY SEARCHING FROM THE LEFT (FROM #8)
C... WITH NOTHING IN THE PREVIOUS LINE, THE FIRST SEARCH POINT IS
C... #4. AFTER A POINT IS FOUND, SEARCH FOR THE NEXT POINT BY STARTING
C... AT (PREVIOUS SEARCH # + 4 +1) OR (P.S.# - 4 + 1) IF P.S.# >= 4.
C
C
110  IPSRC=4
ISRC=IPSRC
PERMCT=0
ORTHO=0
DIAG=0
IYMIN=IROW
IYMAX=IROW
IXMIN=ICOL
IXMAX=ICOL
IYST=IROW
IXST=ICOL
C
120  IF(IYST+COORD(1, ISRC) .LT. IRYTOP .OR. IYST+COORD(1,
    CISRC) .GT. IRYBOT) GOTO 125          !CHECK IF OUT OF ROI
    IF(IXST+COORD(2, ISRC) .LT. IRXTOP .OR. IXST+COORD(2,
    CISRC) .GT. IRXBOT) GOTO 125
    IF(THRESH(IXST+COORD(2, ISRC), IYST+COORD(1, ISRC)) .NE. 0) GOTO 160
125  ISRC=ISRC+1
    IF(IADJCT .EQ. 0) ISRC=ISRC+1          !ONLY CHECK ORTHO POINTS
    IF(ISRC .GT. 8) ISRC=ISRC-8
    IF(ISRC .NE. IPSRC) GOTO 120
C...  POINT IS ISOLATED. PERIMETER IS 1 POINT
    PERMCT=1
    GOTO 200
C
160  IF(JMOD(ISRC, 2) .EQ. 0) THEN        !DETERMINE IF ORTHO OR DIAG
    ORTHO=ORTHO+1
    ELSE
        DIAG=DIAG+1
    END IF
    IYST=IYST+COORD(1, ISRC)              !MOVE TO NEW POINT
    IXST=IXST+COORD(2, ISRC)
    IF(THRESH(IXST, IYST) .GT. 0) THEN
        PERMCT=PERMCT+1 !COUNT 'NEW' POINT
        THRESH(IXST, IYST)= -THRESH(IXST, IYST) !NEGATE POINT AS FLAG
    END IF
C
C...  AT THIS POINT, YOU MUST CHECK IF ALL SURROUNDING POINTS HAVE BEEN
C...  COUNTED. SEARCH AND SEE IF ALL SURROUNDING POINT OF THE PRESENT
C...  MICROSTRUCTURE HAVE BEEN SET TO -VALUE
C
    IF(IYST .EQ. IROW .AND. IXST .EQ. ICOL) THEN

```

```

IADJ_COUNT=0
IADJ_NOT_COUNT=0
ISKIP=2
IF(IADJCT .EQ. 1) ISKIP=1
DO 170 ICHK=ISKIP,8,ISKIP
  IF(IYST+COORD(1,ICHK) .LT. IRYTOP .OR. IYST+COORD(1,
  CCHK) .GT. IRYBOT) GOTO 170 !CHECK IF OUT OF ROI
  IF(IXST+COORD(2,ICHK) .LT. IRXTOP .OR. IXST+COORD(2,
  CCHK) .GT. IRXBOT) GOTO 170
  IF(THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK)) .LT. 0) THEN
    IADJ_COUNT=IADJ_COUNT+1
    ICOUNT(ICOUNT)=ICHK
  END IF
  IF(THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK)) .GT. 0)
  C IADJ_NOT_COUNT=IADJ_NOT_COUNT+1
170  CONTINUE
  IF(IADJ_NOT_COUNT .EQ. 0) GOTO 200 !BACK AT 1ST POINT
  IF(IADJ_COUNT .EQ. 1) GOTO 180 !CONTINUE SEARCH
  DO 172 JJ=1,IADJ_COUNT-1
  DO 172 KK=2,IADJ_COUNT
    IDIF=IABS(ICOUNT(JJ)-ICOUNT(KK))
    IF(IDIF .EQ. 1 .OR. IDIF .EQ. 7) GOTO 172
    GOTO 200 !SEPERATED BY TWO, BACK AT 1ST POINT
172  CONTINUE
  GOTO 180
END IF
C
C
C ISKIP=2
C IF(IADJCT .EQ. 1) ISKIP=1
C DO 170 ICHK=ISKIP,8,ISKIP
C IF(IYST .LT. 60) GOTO 173
C TYPE 172,IXST,IYST,ICHK,THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK))
C172  FORMAT(' IXST = ''15' IYST = ''15' ICHK = ''14' THRESH = ''17')
C173  IF(THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK)) .EQ. 0) THEN
C     IZRFLG=0
C     GOTO 170
C   END IF
C   IF(THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK)) .LT. 0) IZRFLG=1
C   IF(THRESH(IXST+COORD(2,ICHK),IYST+COORD(1,ICHK)) .GT. 0
C     C AND. IZRFLG .EQ. 0) GOTO 180
C170  CONTINUE
C   GOTO 200 !BACK AT 1ST POINT
C   END IF
C
C... SET RANGE VALUES
180  IF(IYST .LT. IYMIN) IYMIN=IYST
    IF(IYST .GT. IYMAX) IYMAX=IYST
    IF(IXST .LT. IXMIN) IXMIN=IXST
    IF(IXST .GT. Ixmax) IXMAX=IXST
C... CALCULATE WHERE TO LOOK NEXT
    IF(1SRC .LE. 4) THEN
      IPSRC=ISRC+4
    ELSE
      IPSRC=ISRC-4
    END IF
    IPSRC=IPSRC+2
    IF(1SRC .GT. B) IPSRC=IPSRC-B
    ISRC=IPSRC
  
```

```

        GOTO 120
C
C...      CALCULATE PERIMETER DISTANCE
C
200      PERIM=(ORTHO+(DIAG*SQRTWO))*SIZPI*           !NORMALIZED TO MICRONS
C
C...      SECOND PASS THROUGH RANGE THAT CONTAINS MICROSTRUCTURE.
C...      DETERMINE MAX HORIZONTAL AND VERTICAL DIAMETERS
C
        IMAX_Y=0
        IMAX_X=0
        DO 320 ICOL=IXMIN,IXMAX                      !CALCULATE Y-MAX DIAMETER
        IST=0
        DO 300 IROW=IYMIN,IYMAX
        IF(IABS(THRESH(ICOL,IROW)) .EQ. IMST) THEN
          IF(IST .EQ. 1) GOTO 290
          IST=1
          ISTLC=IROW
          CONTINUE
290      ELSE
          IF(IST .EQ. 0) GOTO 295
          IF((IROW-1)-ISTLC .GT. IMAX_Y) IMAX_Y=IROW-1-ISTLC
          IST=0
295      CONTINUE
        END IF
300      CONTINUE
        IF(IST .EQ. 0) GOTO 320
        IF((IROW-1)-ISTLC .GT. IMAX_Y) IMAX_Y=IROW-1-ISTLC
320      CONTINUE
C
C...      THIRD PASS THROUGH RANGE THAT CONTAINS MICROSTRUCTURE. DETERMINE
C...      GEOMETRIC CENTER, AREA, IOD, ODmax, AND AVERAGE OPTICAL DENSITY.
C
C...      CLEAR ALL ARRAYS
        IAREA=0
        IYSUM=0
        IXSUM=0
        WYSUM=0.
        WXSUM=0.
        IOD=0
        ODmax=-1
C
        DO 420 IROW=IYMIN,IYMAX
        IST=0
        DO 400 ICOL=IXMIN,IXMAX
        IF(IABS(THRESH(ICOL,IROW)) .EQ. IMST) THEN
          IIMG=IMAGE(ICOL,IROW)
          IF(IST .EQ. 1) GOTO 390
          IST=1
          ISTLC=ICOL
390      IAREA=IAREA+1
          IYSUM=IYSUM+IROW
          IXSUM=IXSUM+ICOL
          IOD=IOD+IIMG
          WYSUM=WYSUM+FLOAT(IROW*IIMG)
          WXSUM=WXSUM+FLOAT(ICOL*IIMG)
          IF(IIMG .GT. ODmax) ODmax=IIMG
        ELSE
          IF(IST .EQ. 0) GOTO 395
          IF((ICOL-1)-ISTLC .GT. IMAX_X) IMAX_X=ICOL-1-ISTLC
        END IF
        END IF
        END DO
        END DO
      
```

```

      ISTAT=0
395    CONTINUE
      END IF
400    CONTINUE
      IF(IST .EQ. 0) GOTO 420
      IF((ICOL-1)-ISTLC .GT. IMAX_X) IMAX_X=ICOL-1-ISTLC
420    CONTINUE
      IMAX_Y=IMAX_Y+1
      IMAX_X=IMAX_X+1
      YCENT=FLOAT(IYSUM)/FLOAT(IAREA)           !GEOMETRIC CENTERS
      XCENT=FLOAT(IXSUM)/FLOAT(IAREA)
      YWCENT=WYSUM/FLOAT(IOD)
      XWCENT=WXSUM/FLOAT(IOD)
      AREA=FLOAT(IAREA)*(SIZPIX*SIZPIX)         !NORMALIZED AREA
      AVGOD=FLOAT(IOD)/FLOAT(IAREA)               !AVERAGE OPTICAL DENSITY
      IF(IAREA .EQ. PERMCT) THEN                !NO 'INTERIOR' POINTS
          PERMC=-1
          PERIM=-1.0
      END IF
C
C...   WRITE INFORMATION TO FILE
C
      WRITE(3,800), ISTAT, XCENT, YCENT, XWCENT, YWCENT, AREA, PERMCT,
      CPERIM, IMAX_X, IMAX_Y, IOD, AVGOD, ODmax
800    FORMAT('T6,15,T1B,F7.2,T27,F7.2,T36,F7.2,T45,F7.2,T53,F9.2,
      CT64,15,T74,F8.2,T89,I3,T102,I3,T109,I8,T11B,F7.2,T127,I3)
C
C...   KEEP A RUNNING TOTAL OF ALL STATISTICS FOR THIS THRESHOLD RANGE.
C...   THIS WILL BE WRITTEN TO AN OUTPUT FILE AT COMPLETION OF ALL PARTICLES
C
      XCEN1=XCEN1+XCENT           !X AND Y CENTERS
      YCEN1=YCEN1+YCEN1
      XTCENT=XTCENT+(XCENT*IOD)
      YTCENT=YTCENT+(YCEN1*IOD)
      XWCEN1=XWCEN1+XWCENT       !WEIGHTED X AND Y CENTERS
      YWCEN1=YWCEN1+YWCENT
      IAREAS=IAREAS+IAREA        !TOTAL AREA
      IF(PERMCT .LT. 0) GOTO B20
      PERMC1=PERMC1+PERMCT       !TOTAL PERIMETER COUNT
B20    IF(PERIM .LT. 0) GOTO B30
      PERIM1=PERIM1+PERIM        !TOTAL PERIMETER LENGTH
B30    IMAX_X_SUM=IMAX_X_SUM+IMAX_X
      IMAX_Y_SUM=IMAX_Y_SUM+IMAX_Y
      IODSUM=IODSUM+IOD          !TOTAL IOD
      ODmaxS=ODmaxS+ODmax        !TOTAL ODmax
C
900    CONTINUE
      CLOSE(UNIT=3,DISPOSE='SAVE')
C
C...   CALCULATE 'AVERAGE' OF STATISTICS AND WRITE TO UNIT 2
C
      IF(UNIQUE .LT. 1) THEN
          AUNO=0
          AOUT1=0
          AOUT2=0
          AOUT3=0
          AOUT6=0
          AOUT7=0
          GOTO 950
      END IF

```

```

AUNG=FLOAT(UNIQUE)
AOUT1=FLOAT(IAREAS)/AUNG
AOUT2=XCEN1/AUNG
AOUT3=YCEN1/AUNG
AOUT6=XTCENT/FLOAT(IODSUM)
AOUT7=YTCENT/FLOAT(IODSUM)
950  WRITE(2,920), ITLOW, ITUPR, IAREAS, UNIQUE, AOUT1, AOUT2, AOUT3,
      CADUT6, AOUT7
920  FORMAT(' ', 13, '-.', 13, 6X, 16, 11X, 16, 11X, F9. 2, 5X, F7. 2, 6X, F7. 2,
      C6X, F7. 2, 7X, F7. 2)
C...  STORE STATS FOR SECOND SECTION OF OUTPUT
NUMAVG=NUMAVG+1
IAVG(NUMAVG, 1)=ITLOW
IAVG(NUMAVG, 2)=ITUPR
IF(UNIQUE .LT. 1) THEN
  IAVG(NUMAVG, 3)=0
  IAVG(NUMAVG, 4)=0
  GAVG(NUMAVG, 1)=0.
  GAVG(NUMAVG, 2)=0.
  GAVG(NUMAVG, 3)=0.
  GAVG(NUMAVG, 4)=0.
  GAVG(NUMAVG, 5)=0.
  GAVG(NUMAVG, 6)=0.
  GAVG(NUMAVG, 7)=0.
ELSE
  IAVG(NUMAVG, 3)=PERMC1
  IAVG(NUMAVG, 4)=IODSUM
  GAVG(NUMAVG, 2)=PERIM1/AUNG
  GAVG(NUMAVG, 3)=FLOAT(IMAX_X_SUM)/AUNG
  GAVG(NUMAVG, 4)=FLOAT(IMAX_Y_SUM)/AUNG
  GAVG(NUMAVG, 5)=FLOAT(IODSUM)/AUNG
  GAVG(NUMAVG, 6)=FLOAT(ODmaiS)/AUNG
  GAVG(NUMAVG, 7)=PERIM1
END IF
999  RETURN
END
C
C
C
C
C

```

```
SUBROUTINE CONFM(OUTFIL,EXT)
INCLUDE 'EDC_MSARCOM.FOR'
BYTE OUTFIL(22),LOW(3),UPR(3),EXT(3)

C          DO 700 JPOINT=1,14           !COPY FILENAME
C          IF(FNAME(JPOINT) .EQ. '.') GOTO 710
C          OUTFIL(JPOINT)=FNAME(JPOINT)
700        CONTINUE
710        ENCODE 3,3,LOW).ITLOW
        ENCODE(3,3,UPR).ITUPR
3         FORMAT(I3)
        OUTFIL(JPOINT)=' '
        OUTFIL(JPOINT+4)=' '
        DO 715 J=1,3
        IF(LOW(J) .EQ. ' ') LOW(J)='0'
        IF(UPR(J) .EQ. ' ') UPR(J)='0'
        OUTFIL(JPOINT+J)=LOW(J)
        OUTFIL(JPOINT+J+4)=UPR(J)
715      CONTINUE
        OUTFIL(JPOINT+8)='.'
        OUTFIL(JPOINT+9)=EXT(')
        OUTFIL(JPOINT+10)=EXT(2)
        OUTFIL(JPOINT+11)=EXT(3)

C          RETURN
C          END
C
C
C
C
```

```

SUBROUTINE WRTINF
INCLUDE 'EDC_MSARCOM.FOR'
BYTE BOLD(4), ATTOFF(4), REVERS(4), BLINK(4)
DATA BOLD/27, 91, 49, 109/           !ESC [ 1 m
DATA ATTOFF/27, 91, 48, 109/          !ESC [ 0 m
DATA REVERS/27, 91, 55, 109/          !ESC [ 7 m
DATA BLINK/27, 91, 53, 109/          !ESC [ 5 m

C
ITOP=4

C
CALL VTCURP(ITOP, 11)               !FILENAME
TYPE 100, FNAME
CALL VTCURP(ITOP+4, 10)              !LOWER THRESHOLD
TYPE 140, ITLOW
CALL VTCURP(ITOP+4, 27)              !UPPER THRESHOLD
TYPE 140, ITUPR
CALL VTCURP(ITOP+6, 15)              !LOWER_INCR
TYPE 140, ITLINC
CALL VTCURP(ITOP+6, 32)              !UPPER_INCR
TYPE 140, ITUINC
CALL VTCURP(ITOP+4, 48)              !ROWS
TYPE 140, IRROW
CALL VTCURP(ITOP+4, 69)              !COLUMNS
TYPE 140, IRCOL
CALL VTCURP(ITOP+5, 49)              !X-TOP
TYPE 140, IRXTOP
CALL VTCURP(ITOP+5, 67)              !Y-TOP
TYPE 140, IRYTOP
CALL VTCURP(ITOP+6, 52)              !X-BOTTOM
TYPE 140, IRXBOT
CALL VTCURP(ITOP+6, 70)              !Y-BOTTOM
TYPE 140, IRYBOT

C
CALL VTCURP(ITOP+5, 22)              !THRESHOLD INCREMENT
IF(ITINCR .EQ. 1) THEN
  TYPE 150
ELSE
  TYPE 160
END IF

C
CALL VTCURP(ITOP+8, 21)              !ADJACENCY CRITERIA
IF(IADJCT .EQ. 1) THEN
  TYPE 170
ELSE
  TYPE 180
END IF

C
CALL VTCURP(ITOP+9, 18)              !PIXEL DIMENSION
IF(IPIXFG .EQ. 1) THEN
  TYPE 190
ELSE
  TYPE 200, SIZPIX
END IF

C
IF(STATUS .EQ. 1) THEN              !PROGRAM STATUS
  TYPE 230, ATTOFF
  CALL VTCURP(ITOP+11, 17)
  TYPE 210
ELSE
  TYPE 230, BOLD

```

```

        TYPE 230, REVERS
        TYPE 230, BLINK
        CALL VTCURP(1TOP+11, 17)
        TYPE 220
        TYPE 230, ATTOFF

END IF

C           FORMAT('+', 14A1)
100        FORMAT('+', I1)
120        FORMAT('+', I3)
140        FORMAT('+'ENABLED ')
150        FORMAT('+'DISABLED')
170        FORMAT('+'ORTHOGONAL/DIAGONAL')
180        FORMAT('+'ORTHOGONAL
190        FORMAT('+'*****')
200        FORMAT('+', F7.3)
210        FORMAT('+'USER-INPUT')
220        FORMAT('+'EXECUTING ')
230        FORMAT('+', 4A1)

C
C           RETURN
END

C
C
C
C
```

```
SUBROUTINE VTCURP(LINE, COL)
C... PLACE CURSOR AT LINE & COL OF SCREEN
C
IMPLICIT INTEGER*2(A-Z)
BYTE CP(8) !HORIZONTAL & VERTICAL CURSOR POSITION
DATA CP/27, 91, 48, 48, 59, 48, 48, 72/ !ESC [ 0 0 ] 0 0 H
ENCODE(2,2,CP(3))LINE
ENCODE(2,2,CP(6))COL
2 FORMAT(12)
IF (CP(3) .EQ. ' ') CP(3) = '0'
IF (CP(6) .EQ. ' ') CP(6) = '0'
TYPE 10, CP
10 FORMAT('$'BA1)
C
RETURN
END
C
C
C
C
```

```
SUBROUTINE CLRTXT
BYTE COMMAND(4)
DATA COMMAND/27, 91, 50, 74/
C
100   TYPE 100,COMMAND
      FORMAT('+'4A1)
      RETURN
      END
C
C
C
C
C
SUBROUTINE CLRLIN(ITOP,IBOT)
BYTE MOVE(7),ERASE(4)
DATA MOVE/27, 91, 0, 0, 59, 49, 72/           !ESC [ P1 : 1 H
DATA ERASE/27, 91, 50, 75/                      !ESC [ 2 K
C
DO 200 J=ITOP, IBOT
ILINE=J
MOVE(3)='0'
IF(ILINE .GT. 9) MOVE(3)=(ILINE/10)+48
MOVE(4)=JMOD(ILINE, 10)+48
TYPE 100,MOVE          !MOVE CURSOR TO LINE
TYPE 100,ERASE          !ERASE ALL OF LINE
100   FORMAT('+'7A1)
200   CONTINUE
      RETURN
      END
C
C
C
C
C
SUBROUTINE CLRSCR
BYTE INIT(3),END(2)
DATA INIT/27, 80, 112/                         !ESC P P
DATA END/27, 92/                                !ESC \
C
100   TYPE 100,INIT,END
      FORMAT('+'3A1, 'S(E)', 2A1)
      RETURN
      END
C
C
C
C
C
```

```

SUBROUTINE VTDBBLE(TEXT,NCHARS,LINE,COL,VID)
C
C... DOUBLE WIDTH VT TERMINAL TEXT PROGRAM
C
C
C CALLING VARIABLES:
C
C     TEXT - input string of text
C     NCHARS - input number of characters of the text [0-40]
C             if nchars = 0, simply set the existing text to double width
C     LINE - input line of text [ 1 - 23 ]
C     COL - input starting column of text [ 1 - 40 ]
C     VID - normal (0) or reverse (1) video
C
C IMPLICIT INTEGER*2(A-Z)
BYTE DW(3),TEXT(40),CP(8),Rv(4),Nv(3)
DATA CP/27,91,4B,4B,59,4B,4B,72/ !CURSOR POSITION
DATA DW/27,35,54/ !DOUBLE WIDTH SINGLE HEIGHT
DATA RV/27,91,55,109/ !ESC [ 7 m REVERSE VIDEO
DATA NV/27,91,109/ !ESC [ m NORMAL VIDEO
IF (LINE .LT. 1) LINE = 1 !VT SCREEN LINES FROM 1
IF (LINE .GT. 23) LINE = 23 ! TO 23
IF (COL .LT. 1) COL = 1
IF (COL .GT. 40) COL = 40
IF (NCHARS + COL .GT. 41) NCHARS = 41 - COL
ENCODE(2,2,CP(3)) LINE
ENCODE(2,2,CP(6)) COL
2 FORMAT(I2)
IF (CP(3) .EQ. ' ') CP(3) = '0' !ZERO FILL 1 DIGIT NUMBERS
IF (CP(6) .EQ. ' ') CP(6) = '0'
IF (NCHARS .LE. 0) THEN !make existing text double width
    TYPE 10,CP,DW
    RETURN
END IF
IF (VID .EQ. 1) THEN
    TYPE 10,CP,DW,Rv,(Text(i),i=1,Nchars),Nv !reverse video text
ELSE
    TYPE 10,CP,DW,Nv,(TEXT(I),I=1,NCHARS) !normal video text
END IF
10 FORMAT('$',58A1)
RETURN
END
C
C
C
C
C

```

```

C
C
C
C      ASYNCHRONOUS KEYBOARD INPUT USING SYSTEM SERVICES QIO
C      WITH WAIT FOR EVENT FLAG TO PREVENT POLLING.
C
C.....  

C
C      FUNCTION IESC ( CHAR )
C
C      IMPLICIT INTEGER*2 ( A - Z )
C      LOGICAL*1 FLAG
C      INTEGER*2 IESC_EFN
C      INTEGER*2 IO$B(4)
C      INTEGER*2 IESC_CHAN
C      INTEGER*2 NEXT(4)
C      BYTE FIRST_CHAR
C      BYTE LAST_TWO_CHARS(2)
C
C      CHARACTER*2 TERMINAL / 'TT' /
C
C      DATA      FLAG / .FALSE. /
C
C      INCLUDE   '( $IODEF )'           !DEFINE SYMBOLIC NAMES FOR I/O FUNCTION
C                                         CODE IO$READVBLK AND ID$M_NOECHO, THE
C                                         CODE MODIFIER.
C
C.....  

C
CHAR = 0
IESC = 0
IESC_EFN = 2
IF (FLAG) GO TO 10
ISTAT = SYS$ASSIGN (TERMINAL, IESC_CHAN, ,)
IF ( .NOT. ISTAT ) CALL LIB$STOP ( XVAL (ISTAT) )
FLAG = .TRUE.
10 IO_FUNC = IO$READVBLK .OR. ID$M_NOECHO .OR. ID$M_ESCAPE
IO_TEST = IO$SENSEMODE .OR. ID$M_TYPEAHDCNT
ISTAT = SYS$QIOW(XVAL(IESC_EFN),          ! READ A CHARACTER
1             XVAL(IESC_CHAN),          ! WHEN KEYED.
2             XVAL(IO_FUNC),          !
3             XREF(IO$B),            !
4             ,                      !
5             ,                      !
6             XREF(FIRST_CHAR),      !
7             XVAL(1),              !
8             ,                      !
9             ,                      !
1             )                    !
C
C      IF (.NOT. ISTAT ) CALL LIB$STOP (XVAL(ISTAT)) ! STOP IF QIO NOT SUCCESS
CHAR = FIRST_CHAR
ISTAT = SYS$QIOW(XVAL(IESC_EFN),          ! HOW MANY MORE CHARACTERS
1             XVAL(IESC_CHAN),
2             XVAL(IO_TEST),
3             XREF( IO$B ) );

```

```
4          '
5          XREF(NEXT),.... )
6
C      IF( NEXT(1) .EQ. 0 )  RETURN
C      ISTAT = SY$QIOW(%VAL(IESC_EFN),
C                         %VAL(IESC_CHAN),
C                         %VAL(IESC_FUNC),
C                         %REF(    IOSB),
C
C                         '
C                         XREF(LAST_TWO_CHARS),%VAL(2),.... )
C
C      CHAR = LAST_TWO_CHARS(2)
C      IESC = 1
C
C      WRITE (6,100) IESC, IDUM
CC100  FORMAT (6X,'BYTE COUNT: ',I4, ' FIRST CHARACTER: ',A1 )
C
20      RETURN
      END
C
C
C
C
C
```

```
SUBROUTINE SCROLL(ITOP,IBOT)
C
C...   SET SCROLL REGION TO ONE DEFINED BY ITOP,IBOT.  THEN MOVE
C...   CURSOR TO 'HOME', TOP LINE OF SCROLL REGION.
C
C     BYTE INIT(2),STRING(6)
C     DATA INIT/27,91/           !ESC [
C
C     STRING(1)=(ITOP/10)+48
C     STRING(2)=(ITOP-((STRING(1)-48)*10))+48
C     STRING(3)=' '
C     STRING(4)=(IBOT/10)+48
C     STRING(5)=(IBOT-((STRING(4)-48)*10))+48
C     STRING(6)='r'
C
C     TYPE 10,"33,(STRING(J),J=1,6)
10    FORMAT('+',A1,'[',6A1)
TYPE 20,INIT
20    FORMAT('+'2A1,'?6h')
RETURN
END
```